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(54) Nucleotide sequence of the mycoplasma genitalium genome, fragments thereof, and uses thereof

(57) The present invention provides the nucleotide sequence of the entire genome of *Mycoplasma genitalium*, SEQ ID NO:1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies protein encoding fragments of the genome, and identifies, by position relative to two (2) genes known to flank the origin of replication, any regulatory elements which modulate the expression of the protein encoding fragments of the *Mycoplasma genitalium* genome.

Description

Cross-Reference to Related Applications

This application is a continuation-in-part of application nos.08/488,018 and 08/473,545, both filed June 7, 1995, and both of which are hereby incorporated by reference.

Background of the Invention

Statement as to Rights to Inventions Made Under Federally-Sponsored Research and Development

Part of the work performed during development of this invention utilized U.S. Government funds. The U.S. Government may have certain rights in the invention -DE-FC02-95ER61962.A000; NP-838C; NIH-AI08998, AI33161, and HL19171.

Field of the Invention

The present invention relates to the field of molecular biology. The invention discloses compositions comprising the nucleotide sequence of *Mycoplasma genitalium*, fragments thereof, and its use in medical diagnostics, therapies and pharmaceutical development.

Related Background Art

Mycoplasmas are the smallest free-living bacterial organisms known (Colman, S.D. *et al., Mol. Microbiol. 4:*683-687 (1990)). Mycoplasmas are thought to have evolved from higher gram-positive bacteria through the loss of genetic material (Bailey, C.C. *et al., J. Bacteriol. 176:*5814-5819 (1994)). *Mycoplasma genitalium (M genitalium)* is widely considered to be the smallest self-replicating biological system, as the molecular size of its genome has been shown to be only 570-600kp (Pyle, L.E. *et al., Nucleic Acids Res. 16(13):*6015-6025 (1988); Peterson, S.N. *et al., J. Bacteriol. 175:*7918-7930(1993)). All mycoplasmas lack a cell wall and have small genomes and a characteristically low G+C content (Razin, S., *Microbiol. Rev. 49(4):*419-455 (1985); Peterson, S.N. *et al., J. Bacteriol. 175:*7918-7930(1993)). Some mycoplasmas, including *M genitalium*, have a specialized codon usage, whereby UGA encodes tryptophan rather than serving as a stop codon (Inamine, J.M. *et al., J. Bacteriol. 172:*504-506 (1990); Tanaka, J.G. *et al., Nucleic Acids Res. 19:*6787-6792 (1991); Yamao, F.A. *et al., Proc. Natl. Acad Sci. USA 82:*2306-2309 (1985)).

Mycoplasmas are widely known to be significant pathogens of humans, animals, and plants (Bailey, C.C. *et al., J. Bacteriol. 176:*5814-5819 (1994)). The metabolic systems of mycoplasmas indicate that they are generally biosynthetically deficient, and thus depend on the microenvironment of the host by characteristically adhering to host cells in order to obtain essential precursor molecules, *i.e.*, amino acids, fatty acids and sterols etc. (Baseman, J.B., 1987. *Mycoplasma Cell Membranes*, Vol. 20. The Plenum Press, New York, NY).

In particular, *M. genitalium*, a newly discovered species, is a pathogenic etiological agent first isolated in 1980 from the urethras of human males infected with non-gonococcal urethritis (Tully, J. G. *et al., Lancet 1:*1288-1291 (1981); Tully, J.G., *et al., Int. J. Syst. Bacteriol. 33:*387-396 (1983)). *M. genitalium* has also been identified in specimens of pneumonia patients as a co-isolate of *Mycoplasma pneumoniae* (Baseman, J.B. *et al., J. Clin. Microbiol.* 26:2266-2269 (1988)). *M. genitalium* opportunistic infection has often been observed in individuals infected with human immunodeficiency virus type 1 (HIV-1) (Lo, S.-C. *et al., Amer. J. Trop. Med. Hyg. 41:*601-616 (1989); Lo, S.-C. *et al., Amer. J. Trop. Med. Hyg. 41:*601-616 (1989); Sasaki, Y. *et al., AIDS Res. Hum. Retrov. 9(8):*775-780 (1993)). Mycoplasmas can also induce various cytokines, including tumor necrosis factor, which may enhance HIV replication (Chowdhury, I.H. *et al., Biochem. Biophys. Res. Commun. 170:*1365-1370 (1990)).

A high amino acid homology exists between the attachment protein of *M. genitalium* and the aligned proteins of several human Class II major histocompatibility complex proteins (HLA), suggesting that *M. genitalium* infection may play an important role in triggering autoimmune mechanisms, thereby aggravating the immunodeficiency characteristics of acquired immune deficiency syndrome (AIDS) (Montagnier, L. *et al., C.R. Acad. Sci. Paris 311(3):*425-430 (1990); Root-Bernstein, R.S. *et al., Res. Immunol. 142:*519-523 (1991); Bisset, L.R. *Autoimmunity 14:*167-168 (1992)). A diagnostic immunoassay for detecting *M. genitalium* infection using monoclonal antibodies specific for some *M. genitalium* antigens has been developed. Baseman, J.B. *et al.*, U.S. Pat. No. 5,158,870.

Due to its diminutive genomic size, *M. genitalium* provides a useful model for determining the minimum number of genes and protein products necessary for a host-independent existence. *M. genitalium* expresses a characteristically low number of base-pairs and low G+C content, which along with its UGA tryptophan codon, has hampered sequencing efforts by conventional techniques (Razin, A., *Microbiol. Rev. 49(4):*419-455 (1985); Colman, S.D. *et al., Gene 87:*91-96 (1990); Dybvig, K. 1992. *Gene Transfer In:* Maniloff, J. (ed.) *Mycoplasmas: Molecular Biology and Pathogenesis.,* *Am. Soc. Microbiol.* Washington, D.C., pp.355-362)). *M. genitalium* possesses a single circular chromosome (Colman, S.D. *et al., Gene 87:*91-96 (1990); Peterson, S.N. *et al., J. Bacteriol. 175:*7918-7930 (1993)). The characterization of the genome of *M. genitalium* has also been hampered by the lack of auxotrophic mutants and by the lack of a system for genetic exchange, precluding reverse genetic approaches. Thus, the sequencing of the *M. genitalium* genome would enhance the understanding of how *M. genitalium* causes or promotes various invasive or immunodeficiency diseases and to how best to medically combat mycoplasma infection.

Prior attempts at characterizing the structure and gene arrangement of the chromosomes of mycoplasmas using pulsed-field gel electrophoretic methods (Pyle, L.E. *et al., Nucleic Acids Res. 16(13):*6015-6025 (1988); Neimark, H.C. *et al., Nucleic Acids Res. 18(18):*5443-5448 (1990)), indicated that mycoplasmas have genomes ranging widely in size. Southern blot hybridization of digested DNAs of *M. genitalium* compared to the well-known human pathogen, *M. pneumoniae,* indicated overall low homology values of approximately 6-8% (Yogev, D. *et al., Int. J. Syst. Bacteriol. 36(3):*426-430 (1986)). However, high homologies have been reported between the adhesin genes of *M. genitalium* and *M. pneumoniae* (Dallo, S.F. *et al., Microbial Path. 6:*69-73 (1989)). Initial studies at characterizing the genome of *M. genitalium* by comparison to the well-known *M. pneumoniae* species, indicated that both species have three (3) rRNA genes clustered together in a chromosomal segment of about 5kb and form a single operon organized in classical procaryotic fashion, but differences exist between their respective restriction sites (Yogev, D. *et al., Int. J. Syst. Bacteriol. 36(3):*426-430 (1986)).

Restriction enzyme mapping of *M. genitalium* indicates that the genome is approximately 600kb. Several genes have also been mapped, including the single ribosomal operon, and the gene encoding the MgPa cytadhesion protein (Su, C.J. *et al., J. Bacteriol. 172:*4705-4707 (1990); Colman, S.D. *et al., Mol. Microbiol. 4(4):*683-687 (1990)). The entire restriction map of the genome of *M. genitalium* has also been cloned in an ordered library of 20 overlapping cosmids and one λ clone (Lucier, T.S. *et al., Gene 150:*27-34 (1994)).

An initial study using random sequencing techniques to characterize the *M. genitalium* genome resulted in forty-four (44) random clones being partially sequenced; several long open reading frames were also found (Peterson, S.N. *et al., Nucleic Acids Rev. 19:*6027-6031 (1991)). Subsequent work using random sequencing of 508 random nonidentical clones has allowed sequence information to be compiled for approximately seventeen percent (17%) (100,993 nucleotides) of the *M. genitalium* genome (Peterson, S.N. *et al., J. Bacteriol. 175:*7918-7930 (1993)). Sequence information indicates that the diminutive genome of *M. genitalium* contains numerous genes involved in various metabolic processes. The genome is estimated to encode approximately 390 proteins, indicating that *M. genitalium* makes very efficient use of its limited amount of DNA (Peterson, S.N. *et al., J. Bacteriol. 175:*7918-7930 (1993)).

Several studies have been undertaken to sequence and characterize individual genes identified in *M. genitalium.* In particular, the medically important aspects of *M. genitalium* have helped to direct interest to those genes which determine the degree of infectivity and the virulence characteristics of the organism. The nucleotide sequence and deduced amino acid sequence for the MgPa adhesin gene, *i.e.*, the gene encoding the surface cytadhesion protein of *M. genitalium*, indicates that the complete gene contains 4,335 nucleotides coding for a protein of 159,668 Da. (Dallo, S.F. *et al., Infect. Immun. 57(4):*1059-1065 (1989)). Furthermore, subsequent nucleotide sequencing of the *M. genitalium* MgPa adhesin gene revealed the specific codon order for this important gene (Inamine, J.M. *et al., Gene 82:*259-267 (1989)). The MgPa adhesin gene also has been shown to express restriction fragment length polymorphism (Dallo, S.F. *et al., Microbial Path. 10:*475-480 (1991)). Nucleotide homology to the well-known highly conserved procaryotic origin-of-replication gene (*gyrA*) was noted for *M. genitalium* (Bailey, C.C. *et al., J. Bacteriol. 176:*5814-5819 (1994)). The highly conserved procaryotic elongation factor, Tu, encoded by the *tuf* gene, has been noted and sequenced for *M. genitalium*, and was found to contain an open reading frame encoding a protein of approximately 393 amino acids (Loechel, S. *et al., Nucleic Acids Res. 17(23):*10127 (1989)). The *tuf* gene of *M. genitalium* has also been determined to use a signal other than a Shine-Delgarno (ribosomal binding site) sequence preceding the initiation codon (Loechel, S. *et al., Nucleic Acids Res. 19:*6905-6911 (1991)).

Summary of the Invention

The present invention is based on the sequencing of the *Mycoplasma genitalium* genome. The primary nucleotide sequence which was generated is provided in SEQ ID NO:1.

The present invention provides the generated nucleotide sequence of the *Mycoplasma genitalium* genome, or a representative fragment thereof, in a form which can be readily used, analyzed, and interpreted by a skilled artisan. In one embodiment, present invention is provided as a contiguous string of primary sequence information corresponding to the nucleotide sequence depicted in SEQ ID NO:1.

The present invention further provides nucleotide sequences which are at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1.

The nucleotide sequence of SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence which is at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 may be provided in a variety of mediums to facilitate its use. In one application of this embodiment, the sequences of the present invention are recorded on computer readable media. Such media includes, but is not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

The present invention further provides systems, particularly computer-based systems which contain the sequence information herein described stored in a data storage means. Such systems are designed to identify commercially important fragments of the *Mycoplasma genitalium* genome.

Another embodiment of the present invention is directed to isolated fragments of the *Mycoplasma genitalium* genome. The fragments of the *Mycoplasma genitalium* genome of the present invention include, but are not limited to, fragments which encode peptides, hereinafter open reading frames (ORFs), fragments which modulate the expression of an operably linked ORF, hereinafter expression modulating fragments (EMFs), fragments which mediate the uptake of a linked DNA fragment into a cell, hereinafter uptake modulating fragments (UMFs), and fragments which can be used to diagnose the presence of *Mycoplasma genitalium* in a sample, hereinafter, diagnostic fragments (DFs).

Each of the ORF fragments of the *Mycoplasma genitalium* genome disclosed in Tables 1(a), 1(c) and 2, and the EMF found 5' to the ORF, can be used in numerous ways as polynucleotide reagents. The sequences can be used as diagnostic probes or diagnostic amplification primers for the presence of a specific microbe in a sample, for the production of commercially important pharmaceutical agents, and to selectively control gene expression.

The present invention further includes recombinant constructs comprising one or more fragments of the *Mycoplasma genitalium* genome of the present invention. The recombinant constructs of the present invention comprise vectors, such as a plasmid or viral vector, into which a fragment of the *Mycoplasma genitalium* has been inserted.

The present invention further provides host cells containing any one of the isolated fragments of the *Mycoplasma genitalium* genome of the present invention. The host cells can be a higher eukaryotic host such as a mammalian cell, a lower eukaryotic cell such as a yeast cell, or can be a procaryotic cell such as a bacterial cell.

The present invention is further directed to isolated proteins encoded by the ORFs of the present invention. A variety of methodologies known in the art can be utilized to obtain any one of the proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. In an alternative method, the protein is purified from bacterial cells which naturally produce the protein. Lastly, the proteins of the present invention can alternatively be purified from cells which have been altered to express the desired protein.

The invention further provides methods of obtaining homologs of the fragments of the *Mycoplasma genitalium* genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. Specifically, by using the nucleotide and amino acid sequences disclosed herein as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

The invention further provides antibodies which selectively bind one of the proteins of the present invention. Such antibodies include both monoclonal and polyclonal antibodies.

The invention further provides hybridomas which produce the above-described antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

The present invention further provides methods of identifying test samples derived from cells which express one of the ORF of the present invention, or homolog thereof. Such methods comprise incubating a test sample with one or more of the antibodies of the present invention, or one or more of the DFs of the present invention, under conditions which allow a skilled artisan to determine if the sample contains the ORF or product produced therefrom.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the above-described assays.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the antibodies, or one of the DFs of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of bound antibodies or hybridized DFs.

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents capable of binding to a protein encoded by one of the ORFs of the present invention. Specifically, such agents include antibodies (described above), peptides, carbohydrates, pharmaceutical agents and the like. Such methods comprise the steps of:

(a) contacting an agent with an isolated protein encoded by one of the ORFs of the present invention; and
(b) determining whether the agent binds to said protein.

The complete genomic sequence of *M. genitalium* will be of great value to all laboratories working with this organism and for a variety of commercial purposes. Many fragments of the *Mycoplasma genitalium* genome will be immediately identified by similarity searches against GenBank or protein databases and will be of immediate value to *Mycoplasma* researchers and for immediate commercial value for the production of proteins or to control gene expression. A specific example concerns PHA synthase. It has been reported that polyhydroxybutyrate is present in the membranes of *M. genitalium* and that the amount correlates with the level of competence for transformation. The PHA synthase that synthesizes this polymer has been identified and sequenced in a number of bacteria, none of which are evolutionarily close to *M. genitalium*. This gene has yet to be isolated from *M. genitalium* by use of hybridization probes or PCR techniques. However, the genomic sequence of the present invention allows the identification of the gene by utilizing search means described below.

Developing the methodology and technology for elucidating the entire genomic sequence of bacterial and other small genomes has and will greatly enhance the ability to analyze and understand chromosomal organization. In particular, sequenced genomes will provide the models for developing tools for the analysis of chromosome structure and function, including the ability to identify genes within large segments of genomic DNA, the structure, position, and spacing of regulatory elements, the identification of genes with potential industrial applications, and the ability to do comparative genomic and molecular phylogeny.

Brief Description of the Figures

Figure 1 - EcoRI restriction map of the *Mycoplasma genitalium* genome.

Figure 2 - Block diagram of a computer system 102 that can be used to implement the computer-based systems of present invention.

Figure 3 - Summary of the *Mycoplasma genitalium* sequencing project.

Figure 4 - A circular representation of the *M. genitalium* chromosome. Outer concentric circle: Coding regions on the plus strand for which a gene identification was made. Each coding region location is coded as to role according to the color code in Figure 5. Second concentric circle: Coding regions on the minus strand for which a gene identification was made. Third concentric circle: The direction of transcription on each strand of the chromosome is depicted as a red arrow starting at the putative origin of replication. Fourth concentric circle: Coverage by cosmid and lambda clones (blue). Nineteen cosmid clones and one lambda clone were sequenced from each end to confirm the overall structure of the genome. Fifth concentric circle: The locations of the single ribosomal operon (blue) and the 33 tRNAs. The clusters of tRNAs (trnA, trnB, trnC, trnD and trnE) are indicated by the letters A-E with the number of tRNAs in each cluster listed in parentheses. Sixth concentric circle: Location of the MgPa operon (green) and MgPa repeat fragments (brown).

Figure 5 - Gene map of the *M. genitalium* genome. Predicted coding regions are shown on each strand. The rRNA operon and tRNA genes are shown as a line and as triangles, respectively. Genes are color-coded by the role category as described in the Figure key. Gene identification numbers correspond to those in Table 6. Where possible, three-letter designations are also provided.

Figure 6 - Location of the MgPa repeats in the *M. genitalium* genome. The structure of the MgPa operon (ORF1-MgPa gene-ORF3) in the *M. genitalium* genome is illustrated across the top. In addition to the complete operon, nine repetitive elements which are composites of particular regions of the MgPa operon were found. The coordinates of each repeat in the genome are indicated on the left and right end of each line. The repetitive elements are located directly below those regions in the operon for which there is sequence similarity. The percent of sequence identity between the repeat elements and the MgPa gene ranges from 78%-90%. In some of the repeats, the MgPa-related sequences are separated in the genome by a variable length, A-T rich spacer sequence (indicated in the figure by a line with the length of the spacer indicated in bp). In cases where no spacer sequence is shown, the composites of the operon are co-linear in the genome. In repeats 7 and 9, the order of the sequences in the repeats differs from that in the operon. In these cases, the order of the elements in each repeat in the genome is indicated numerically where element 1 is followed by element 2 which is followed by element 3, etc.

Detailed Description of the Preferred Embodiments

The present invention is based on the sequencing of the *Mycoplasma genitalium* genome. The primary nucleotide sequence which was generated is provided in SEQ ID NO:1. As used herein, the "primary sequence" refers to the nucleotide sequence represented by the IUPAC nomenclature system.

The sequence provided in SEQ ID NO:1 is oriented relative to two genes (DNAA and DNA gyrase) known to flank the origin of replication of the *Mycoplasma genitalium* genome. A skilled artisan will readily recognize that this start/stop point was chosen for convenience and does not reflect a structural significance.

The present invention provides the nucleotide sequence of SEQ ID NO:1, or a representative fragment thereof, in a form which can be readily used, analyzed, and interpreted by a skilled artisan. In one embodiment, the sequence is provided as a contiguous string of primary sequence information corresponding to the nucleotide sequence provided in SEQ ID NO:1.

As used herein, a "representative fragment of the nucleotide sequence depicted in SEQ ID NO:1" refers to any portion of SEQ ID NO:1 which is not presently represented within a publicly available database. Preferred representative fragments of the present invention are *Mycoplasma genitalium* open reading frames, expression modulating fragments, uptake modulating fragments, and fragments which can be used to diagnose the presence of *Mycoplasma genitalium* in sample. A non-limiting identification of such preferred representative fragments is provided in Tables 1(a), 1(c) and 2.

The nucleotide sequence information provided in SEQ ID NO:1 was obtained by sequencing the *Mycoplasma genitalium* genome using a megabase shotgun sequencing method. The nucleotide sequence provided in SEQ ID NO:1 is a highly accurate, although not necessarily a 100% perfect, representation of the nucleotide sequence of the *Mycoplasma genitalium* genome.

As discussed in detail below, using the information provided in SEQ ID NO:1 and in Tables 1(a), 1(c) and 2 together with routine cloning and sequencing methods, one of ordinary skill in the art would be able to clone and sequence all "representative fragments" of interest including open reading frames (ORFs) encoding a large variety of *Mycoplasma genitalium* proteins. In very rare instances, this may reveal a nucleotide sequence error present in the nucleotide sequence disclosed in SEQ ID NO:1. Thus, once the present invention is made available (i.e., once the information in SEQ ID NO:1 and Tables 1(a), 1(c) and 2 have been made available), resolving a rare sequencing error in SEQ ID NO:1 would be well within the skill of the art. Nucleotide sequence editing software is publicly available. For example, Applied Biosystem's (AB) AutoAssembler™ can be used as an aid during visual inspection of nucleotide sequences.

Even if all of the very rare sequencing errors in SEQ ID NO:1 were corrected, the resulting nucleotide sequence would still be at least 99.9% identical to the nucleotide sequence in SEQ ID NO:1.

The nucleotide sequences of the genomes from different strains of *Mycoplasma genitalium* differ slightly. However, the nucleotide sequence of the genomes of all *Mycoplasma genitalium* strains will be at least 99.9% identical to the nucleotide sequence provided in SEQ ID NO:1.

Thus, the present invention further provides nucleotide sequences which are at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 in a form which can be readily used, analyzed and interpreted by the skilled artisan. Methods for determining whether a nucleotide sequence is at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 are routine and readily available to the skilled artisan. For example, the well known **fasta** algothrithm (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA 85*:2444 (1988)) can be used to generate the percent identity of nucleotide sequences.

Computer Related Embodiments

The nucleotide sequence provided in SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 may be "provided" in a variety of mediums to facilitate use thereof. As used herein, provided refers to a manufacture, other than an isolated nucleic acid molecule, which contains a nucleotide sequence of the present invention, i.e., the nucleotide sequence provided in SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1. Such a manufacture provides the *Mycoplasma genitalium* genome or a subset thereof (e.g., a *Mycoplasma genitalium* open reading frame (ORF)) in a form which allows a skilled artisan to examine the manufacture using means not directly applicable to examining the *Mycoplasma genitalium* genome or a subset thereof as it exists in nature or in purified form.

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently know methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of dataprocessor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing the nucleotide sequence of SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul *et al., J. Mol. Biol. 215*:403-410 (1990)) and BLAZE (Brutlag *et al., Comp. Chem. 17*:203-207 (1993)) search algorithms on a Sybase system was used to identify open reading frames

(ORFs) within the *Mycoplasma genitalium* genome which contain homology to ORFs or proteins from other organisms. Such ORFs are protein encoding fragments within the *Mycoplasma genitalium* genome and are useful in producing commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

The present invention further provides systems, particularly computer-based systems, which contain the sequence information described herein. Such systems are designed to identify commercially important fragments of the *Mycoplasma genitalium* genome.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based system are suitable for use in the present invention.

As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the *Mycoplasma genitalium* genome which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are available and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that searches for commercially important fragments of the *Mycoplasma genitalium* genome, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzymic active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

A variety of structural formats for the input and output means can be used to input and output the information in the computer-based systems of the present invention. A preferred format for an output means ranks fragments of the *Mycoplasma genitalium* genome possessing varying degrees of homology to the target sequence or target motif. Such presentation provides a skilled artisan with a ranking of sequences which contain various amounts of the target sequence or target motif and identifies the degree of homology contained in the identified fragment.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify sequence fragments of the *Mycoplasma genitalium* genome. In the present examples, implementing software which implement the BLAST and BLAZE algorithms (Altschul *et al., J. Mol. Biol. 215*:403-410 (1990)) was used to identify open reading frames within the *Mycoplasma genitalium* genome. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer-based systems of the present invention.

One application of this embodiment is provided in Figure 2. Figure 2 provides a block diagram of a computer system 102 that can be used to implement the present invention. The computer system 102 includes a processor 106 connected to a bus 104. Also connected to the bus 104 are a main memory 108 (preferably implemented as random access memory, RAM) and a variety of secondary storage devices 110, such as a hard drive 112 and a removable medium storage device 114. The removable medium storage device 114 may represent, for example, a floppy disk drive, a CD-ROM drive, a magnetic tape drive, etc. A removable storage medium 116 (such as a floppy disk, a compact disk, a magnetic tape, etc.) containing control logic and/or data recorded therein may be inserted into the removable medium storage device 114. The computer system 102 includes appropriate software for reading the control logic and/or the data from the removable medium storage device 114 once inserted in the removable medium storage device 114.

A nucleotide sequence of the present invention may be stored in a well known manner in the main memory 108, any of the secondary storage devices 110, and/or a removable storage medium 116. Software for accessing and processing the genomic sequence (such as search tools, comparing tools, etc.) reside in main memory 108 during execution.

Biochemical Embodiments

Another embodiment of the present invention is directed to isolated fragments of the *Mycoplasma genitalium* genome. The fragments of the *Mycoplasma genitalium* genome of the present invention include, but are not limited to fragments which encode peptides, hereinafter open reading frames (ORFs), fragments which modulate the expression of an operably linked ORF, hereinafter expression modulating fragments (EMFs), fragments which mediate the uptake of a linked DNA fragment into a cell, hereinafter uptake modulating fragments (UMFs), and fragments which can be used to diagnose the presence of *Mycoplasma genitalium* in a sample, hereinafter diagnostic fragments (DFs).

As used herein, an "isolated nucleic acid molecule" or an "isolated fragment of the *Mycoplasma genitalium* genome" refers to a nucleic acid molecule possessing a specific nucleotide sequence which has been subjected to purification means to reduce, from the composition, the number of compounds which are normally associated with the composition. A variety of purification means can be used to generated the isolated fragments of the present invention. These include, but are not limited to methods which separate constituents of a solution based on charge, solubility, or size.

In one embodiment, *Mycoplasma genatalium* DNA can be mechanically sheared to produce fragments of 15-20 kb in length. These fragments can then be used to generate an *Mycoplasma genitalium* library by inserting them into lambda clones as described in the Examples below. Primers flanking, for example, an ORF provided in Table 1(a), 1(c) or 2 can then be generated using nucleotide sequence information provided in SEQ ID NO:1. PCR cloning can then be used to isolate the ORF from the lambda DNA library. PCR cloning is well known in the art. Thus, given the availability of SEQ ID NO:1, Table 1(a), 1(c) and Table 2, it would be routine to isolate any ORF or other representative fragment of the present invention.

The isolated nucleic acid molecules of the present invention include, but are not limited to single stranded and double stranded DNA, and single stranded RNA.

As used herein, an "open reading frame," ORF, means a series of triplets coding for amino acids without any termination codons and is a sequence translatable into protein. Tables 1(a), 1(b), 1(c) and 2 identify ORFs in the *Mycoplasma genitalium* genome. In particular, Table 1(a) indicates the location of ORFs (i.e., the adresses) within the *Mycoplasma genitalium* genome which encode the recited protein based on homology matching with protein sequences from the organism appearing in parentheticals (see the fifth column of Table 1(a)).

The first column of Table 1(a) provides the "UID" (an arbitrary identification number) of a particular ORF. The second and third columns in Table 1(a) indicate an ORFs position in the nucleotide sequence provided in SEQ ID NO:1. One of ordinary skill in the art will recognize that ORFs may be oriented in opposite directions in the *Mycoplasma genitalium* genome. This is reflected in columns 2 and 3.

The fourth column of Table 1(a) provides the accession number of the database match for the ORF. As indicated above, the fifth column of Table 1(a) provides the name of the database match for the ORF.

The sixth column of Table 1(a) indicates the percent identity of the protein encoded for by an ORF to the corresponding protein from the orgaism appearing in parentheticals in the fifth column. The seventh column of Table 1(a) indicates the percent similarity of the protein encoded for by an ORF to the corresponding protein from the organism appearing in parentheticals in the fifth column. The concepts of percent identity and percent similarity of two polypeptide sequences are well understood in the art. For example, two polypeptides 10 amino acids in length which differ at three amino acid positions (e.g., at positions 1,3 and 5) are said to have a percent identity of 70%. However, the same two polypeptides would be deemed to have a percent similarity of 80% if, for example at position 5, the amino acids moieties, although not identical, were "similar" (i.e., possessed similar biochemical characteristics). The eighth column in Table 1(a) indicates the length of the ORF in nucleotides.

Table 1(b) is a list of ORFs that have database matches to previously published *Mycoplasma genitalium* sequences over the full length of the ORF. The talbe headings for Table 1(b) are identical for Table 1(a) with the following two exceptions: (I) The heading for the eighth column in Table 1(a) (i.e., nucleotide length of the ORF) has been replaced with the following in Table 1(b): "Match_info". "Match_info" refers to the coordinates of the match of the ORF and the previously published *Mycoplama genitalium* sequence. For example, "MG002 (1-930 of 930) GB:U09251 (298-1227 of 6140)," indicates that for ORF MG002, which is 930 nucleotides in length, there is a database match to accession number GB:U09251, which has a total length of 6140 nucleotides. The ORF matches this accession from position 298 to 1227. (II) Where an ORF shows homology matches for both a previously published *Mycoplasma genitalium* sequence and a previously published sequence from a different organism, columns 3, 4, 5, and 6 of Table 1(b) respectively provide the accession number, protein name (and organism in parentheticals), percent identity and percent similarity for the "other organism," rather than for the previously published *Mycoplasma genitalium* sequence. (However, in this scenario, the accession number for the *Mycoplasma genitalium* sequence is still provided in column 8.)

Table 1(c) provides ORFs having database matches to previously published *Mycoplasma genitalium* sequences but only over a portion of the ORF. The table headings are the same as above for Table 1(b).

In Tables 1(a), 1(b) and 1(c), unique identifiers are used to identify the recited ORFs, (e.g., "MG123"). In the parent application nos. 08/488,018 and 08/473,545, the recited ORFs are identified unsing the "MORF" identifier. Table 1(d) lists which of the new and old identifiers refer to the same ORF. For example, the first entry in Table 1(d) indicates that the ORF identifed as MG001 in the current application is the same ORF which was previously identified as MORF-20072 in parent applicaiton nos. 08/488,018 and 08/473,545. Similarly, the third entry in Table 1(d) indicates that the ORF identified as MG003 in the current application is the same ORF which was previously identified as MORF-19818 and MORF-20073 in the parent applications.

Table 2 provides ORFs of the *Mycoplasma genitalium* genome which did not elicit a "homology match" with a known sequence from either *M. genitalium* or another organism.

Table 6 classifies each ORF according to its role category (adapted from Riley, M., *Microbiol. Rev.* 57:862 (1992)). The gene identification, the accession number from public archives that corresponds to the best match, the percent amino acid identity, and the length of the match in amino acids is also listed for each entry as above in Tables 1 (a-c). Those genes in *M. genitalium* that also match a gene in *H. influenzae* are indicated by an asterisk (*) For the purposes of Tables 6 and 7 and Figure 4, each of the MgPa repetitive elements has been assigned an MG number, even though there is evidence to suggest that these repeats may not be transcribed.

Table 7 sorts the gene content in *H. influenzae* and *M. genitalium* by functional category. The number of genes in each category is listed for each organism. The number in parentheses indicates the percent of the putatively identified genes devoted to each functional cetegory. For the category of unassigned genes, the percent of the genome indicated in parentheses represents the percent of the total number of putative coding regions.

Further details concerning the algorithms and criteria used for homology searches are provided in the Examples below.

A skilled artisan can readily identify ORFs in the *Mycoplasma genitalium* genome other than those listed in Tables 1(a), 1(b), 1(c) and 2, such as ORFs which are overlapping or encoded by the opposite strand of an identified ORF in addition to those ascertainable using the computer-based systems of the present invention.

As used herein, an "expression modulating fragment," EMF, means a series of nucleotide molecules which modulates the expression of an operably linked ORF or EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs are fragments which induce the expression or an operably linked ORF in response to a specific regulatory factor or physiological event. A review of known EMFs from *Mycoplasma* are described by (Tomb *et al. Gene 104*:1-10 (1991), Chandler, M. S., *Proc. Natl. Acad Sci. USA 89*:1626-1630 (1992).

EMF sequences can be identified within the *Mycoplasma genitalium* genome by their proximity to the ORFs provided in Tables 1(a), 1(b), 1(c) and 2. An intergenic segment, or a fragment of the intergenic segment, from about 10 to 200 nucleotides in length, taken 5' from any one of the ORFs of Tables 1(a), 1(b), 1(c) or 2 will modulate the expression of an operably linked 3' ORF in a fashion similar to that found with the naturally linked ORF sequence. As used herein, an "intergenic segment" refers to the fragments of the *Mycoplasma* genome which are between two ORF(s) herein described. Alternatively, EMFs can be identified using known EMFs as a target sequence or target motif in the computer-based systems of the present invention.

The presence and activity of an EMF can be confirmed using an EMF trap vector. An EMF trap vector contains a cloning site 5' to a marker sequence. A marker sequence encodes an identifiable phenotype, such as antibiotic resistance or a complementing nutrition auxotrophic factor, which can be identified or assayed when the EMF trap vector is placed within an appropriate host under appropriate conditions. As described above, an EMF will modulate the expression of an operably linked marker sequence. A more detailed discussion of various marker sequences is provided below.

A sequence which is suspected as being an EMF is cloned in all three reading frames in one or more restriction sites upstream from the marker sequence in the EMF trap vector. The vector is then transformed into an appropriate host using known procedures and the phenotype of the transformed host in examined under appropriate conditions. As described above, an EMF will modulate the expression of an operably linked marker sequence.

As used herein, an "uptake modulating fragment," UMF, means a series of nucleotide molecules which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described above.

The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence. A review of DNA uptake in *Mycoplasma* is provided by Goodgall, S.H., *et al., J. Bact. 172*:5924-5928 (1990).

As used herein, a "diagnostic fragment," DF, means a series of nucleotide molecules which selectively hybridize to

Mycoplasma genitalium sequences. DFs can be readily identified by identifying unique sequences within the *Mycoplasma genitalium* genome, or by generating and testing probes or amplification primers consisting of the DF sequence in an appropriate diagnostic format which determines amplification or hybridization selectivity.

The sequences falling within the scope of the present invention are not limited to the specific sequences herein described, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO: 1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 with a sequence from another isolate of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another which encodes the same amino acid is expressly contemplated.

Any specific sequence disclosed herein can be readily screened for errors by resequencing a particular fragment, such as an ORF, in both directions (i.e., sequence both strands). Alternatively, error screening can be performed by sequencing correspond polynucleotides of *Mycoplasma genitalium* origin isolated by using part or all of the fragments in question as a probe or primer.

Each of the ORFs of the *Mycoplasma genitalium* genome disclosed in Tables 1(a), 1(b), 1(c) and 2, and the EMF found 5' to the ORF, can be used in numerous ways as polynucleotide reagents. The sequences can be used as diagnostic probes or diagnostic amplification primers to detect the presence of a specific microbe, such as *Mycoplasma genitalium*, in a sample. This is especially the case with the fragments or ORFs of Table 2, which will be highly selective for *Mycoplasma genitalium*.

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al., Nucl. Acids Res. 6*:3073 (1979); Cooney *et al., Science 241*:456 (1988); and Dervan *et al., Science 251*:1360 (1991)) or to the mRNA itself (antisense - Okano, *J. Neurochem. 56*:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988)).

Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

The present invention further provides recombinant constructs comprising one or more fragments of the *Mycoplasma genitalium* genome of the present invention. The recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a fragment of the *Mycoplasma genitalium* has been inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. For vectors comprising the EMFs and UMFs of the present invention, the vector may further comprise a marker sequence or heterologous ORF operably linked to the EMF or UMF. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs, KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

The present invention further provides host cells containing anyone of the isolated fragments of the *Mycoplasma genitalium* genome of the present invention, wherein the fragment has been introduced into the host cell using known transformulation methods. The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a procaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. *et al., Basic Methods in Molecular Biology* (1986)).

The host cells containing one of the fragments of the *Mycoplasma genitalium* genome of the present invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (e.g., an ORF) by nucleotide sequence but, due to the degeneracy of the Genetic Code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs depicted in Tables 1(a), 1(c) and 2.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polpeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level.

"Recombinant," as used herein, means that a polypeptide or protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, e.g., *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern different from that expressed in mammalian cells.

"Nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. Generally, DNA segments encoding the polypeptides and proteins provided by this invention are assembled from fragments of the *Mycoplasma genitalium* genome and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

"Recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. The expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

"Recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extra chromosomally. The cells can be prokaryotic or eukaryotic. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryatic and eukaryotic hosts are described by Sambrook, *et al., in Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may, also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell* 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Recombinant polypeptides and proteins produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

The present invention further includes isolated polypeptides, proteins and nucleic acid molecules which are substantially equivalent to those herein described. As used herein, substantially equivalent can refer both to nucleic acid and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between reference and subject sequences. For purposes of the present invention, sequences having equivalent biological activity, and equivalent expression characteristics are considered substantially equivalent. For purposes of determining equivalence, truncation of the mature sequence should be disregarded.

The invention further provides methods of obtaining homologs from other strains of *Mycoplasma genitalium*, of the fragments of the *Mycoplasma genitalium* genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. As used herein, a sequence or protein of *Mycoplasma genitalium* is defined as a homolog of a fragment of the *Mycoplasma genitalium* genome or a protein encoded by one of the ORFs of the present invention, if it shares significant homology to one of the fragments of the *Mycoplasma genitalium* genome of the present invention or a protein encoded by one of the ORFs of the present invention. Specifically, by using the sequence disclosed herein as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

As used herein, two nucleic acid molecules or proteins are said to "share significant homology" if the two contain regions which process greater than 85% sequence (amino acid or nucleic acid) homology.

Region specific primers or probes derived from the nucleotide sequence provided in SEQ ID NO:1 or from a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 can be used to prime DNA synthesis and PCR amplification, as well as to identify colonies containing cloned DNA encoding a homolog using known methods (Innis *et al., PCR Protocols*, Academic Press, San Diego, CA (1990)).

When using primers derived from SEQ ID NO:1 or from a nucleotide sequence at least 99.9% identical to SEQ ID NO:1, one skilled in the art will recognize that by employing high stringency conditions (e.g., annealing at 50-60°C) only sequences which are greater than 75% homologous to the primer will be amplified. By employing lower stringency conditions (e.g., annealing at 35-37°C), sequences which are greater than 40-50% homologous to the primer will also be amplified.

When using DNA probes derived from SEQ ID NO:1 or from a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 for colony/plaque hybridization, one skilled in the art will recognize that by employing high stringency conditions (e.g., hybridizing at 50-65°C in 5X SSPC and 50% formamide, and washing at 50-65°C in 0.5X SSPC), sequences having regions which are greater than 90% homologous to the probe can be obtained, and that by employing lower stringency conditions (e.g., hybridizing at 35-37°C in 5X SSPC and 40-45% formamide, and washing at 42°C in SSPC), sequences having regions which are greater than 35-45% homologous to the probe will be obtained.

Any organism can be used as the source for homologs of the present invention so long as the organism naturally expresses such a protein or contains genes encoding the same. The most preferred organism for isolating homologs are bacterias which are closely related to *Mycoplasma genitalium*.

Uses for the Compositions of the Invention

Each ORF provided in Table 1(a), 1(b) and 1(c) was assigned to biological role categories adapted from Riley, M., *Microbiology Reviews 57(4)*:862 (1993)). This allows the skilled artisan to determine a use for each identified coding sequence. Tables 1(a), 1(b) and 1(c) further provides an identification of the type of polypeptide which is encoded for by each ORF. As a result, one skilled in the art can use the polypeptides of the present invention for commercial, therapeutic and industrial purposes consistent with the type of putative identification of the polypeptide.

Such identifications permit one skilled in the art to use the *Mycoplasma genitalium* ORFs in a manner similar to the known type of sequences for which the identification is made; for example, to ferment a particular sugar source or to produce a particular metabolite. (For a review of enzymes used within the commercial industry, *see Biochemical Engineering and Biotechnology Handbook* 2nd, eds. Macmillan Publ. Ltd., NY (1991) and Biocatalysts in Organic Syntheses, ed. J. Tramper *et al.*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985)).

1. Biosynthetic Enzymes

Open reading frames encoding proteins involved in mediating the catalytic reactions involved in intermediary and macromolecular metabolism, the biosynthesis of small molecules, cellular processes and other functions includes enzymes involved in the degradation of the intermediary products of metabolism, enzymes involved in central intermediary metabolism, enzymes involved in respiration, both aerobic and anaerobic, enzymes involved in fermentation, enzymes involved in ATP proton motor force conversion, enzymes involved in broad regulatory function, enzymes involved in amino acid synthesis, enzymes involved in nucleotide synthesis, enzymes involved in cofactor and vitamin synthesis, can be used for industrial biosynthesis. The various metabolic pathways present in *Mycoplasma* can be identified based on absolute nutritional requirements as well as by examining the various enzymes identified in Table 1(a), 1(b) and 1(c).

Identified within the category of intermediary metabolism, a number of the proteins encoded by the identified ORFs in Tables 1(a), 1(b) and 1(c) are particularly involved in the degradation of intermediary metabolites as well as non-macromolecular metabolism. Some of the enzymes identified include amylases, glucose oxidases, and catalase.

Proteolytic enzymes are another class of commercially important enzymes. Proteolytic enzymes find use in a number of industrial processes including the processing of flax and other vegetable fibers, in the extraction, clarification and depectinization of fruit juices, in the extraction of vegetables' oil and in the maceration of fruits and vegetables to give unicellular fruits. A detailed review of the proteolytic enzymes used in the food industry is provided by Rombouts *et al.*, *Symbiosis 21*:79 (1986) and Voragen *et al.* in *Biocatalyst in Agricultural Biotechnology*, edited J.R. Whitaker *et al.*, *American Chemical Society Symposium Series 389*:93 (1989)).

The metabolism of glucose, galactose, fructose and xylose are important parts of the primary metabolism of *Mycoplasma*. Enzymes involved in the degradation of these sugars can be used in industrial fermentation. Some of the important sugar transforming enzymes, from a commercial viewpoint, include sugar isomerases such as glucose isomerase. Other metabolic enzymes have found commercial use such as glucose oxidases which produces ketogulonic acid (KGA). KGA is an intermediate in the commercial production of ascorbic acid using the Reichstein's procedure (*see Krueger et al.*, *Biotechnology 6(A)*, Rhine, H.J. *et al.*, eds., Verlag Press, Weinheim, Germany (1984)).

Glucose oxidase (GOD) is commercially available and has been used in purified form as well as in an immobilized form for the deoxygenation of beer. *See* Hartmeir *et al.*, *Biotechnology Letters 1*:21 (1979). The most important application of GOD is the industrial scale fermentation of gluconic acid. Market for gluconic acids which are used in the detergent, textile, leather, photographic, pharmaceutical, food, feed and concrete industry (*see* Bigelis in *Gene Manipulations and Fungi*, Benett, J.W. *et al.*, eds., Academic Press, New York (1985), p. 357). In addition to industrial applications, GOD has found applications in medicine for quantitative determination of glucose in body fluids recently in biotechnology for analyzing syrups from starch and cellulose hydrosylates. *See Owusu et al.*, *Biochem. et Biophysica. Acta. 872*:83 (1986).

The main sweetener used in the world today is sugar which comes from sugar beets and sugar cane. In the field of industrial enzymes, the glucose isomerase process shows the largest expansion in the market today. Initially, soluble enzymes were used and later immobilized enzymes were developed (Krueger *et al.*, *Biotechnology*, *The Textbook of Industrial Microbiology*, Sinauer Associated Incorporated, Sunderland, Massachusetts (1990)). Today, the use of glucose-produced high fructose syrups is by far the largest industrial business using immobilized enzymes. A review of the industrial use of these enzymes is provided by Jorgensen, *Starch 40*:307 (1988).

Proteinases, such as alkaline serine proteinases, are used as detergent additives and thus represent one of the largest volumes of microbial enzymes used in the industrial sector. Because of their industrial importance, there is a large body of published and unpublished information regarding the use of these enzymes in industrial processes. (See Faultman *et al.*,*Acid Proteases Structure Function and Biology*, Tang, J., ed., Plenum Press, New York (1977) and Godfrey *et al.*,*Industrial Enzymes*, MacMillan Publishers, Surrey, UK (1983) and Hepner *et al.*, *Report Industrial Enzymes by 1990*, Hel Hepner & Associates, London (1986)).

Another class of commercially usable proteins of the present invention are the microbial lipases identified in Tables 1(a), 1(b) and 1(c) (see Macrae *et al.*, *Philosophical Transactions of the Chiral Society of London 310*:227 (1985) and Poserke, *Journal of the American Oil Chemist Society 61*:1758 (1984). A major use of lipases is in the fat and oil industry for the production of neutral glycerides using lipase catalyzed inter-esterification of readily available triglycerides. Application of lipases include the use as a detergent additive to facilitate the removal of fats from fabrics in the course of the washing procedures.

The use of enzymes, and in particular microbial enzymes, as catalyst for key steps in the synthesis of complex organic molecules is gaining popularity at a great rate. One area of great interest is the preparation of chiral intermediates. Preparation of chiral intermediates is of interest to a wide range of synthetic chemists particularly those scientists involved with the preparation of new pharmaceuticals, agrochemicals, fragrances and flavors. (See Davies *et al.*, *Recent Advances in the Generation of Chiral Intermediates Using Enzymes*, CRC Press, Boca Raton, Florida (1990)). The following reactions catalyzed by enzymes are of interest to organic chemists: hydrolysis of carboxylic acid esters, phosphate esters, amides and nitriles, esterification reactions, trans-esterification reactions, synthesis of amides, reduction of alkanones and oxoalkanates, oxidation of alcohols to carbonyl compounds, oxidation of sulfides to sulfoxides, and carbon bond forming reactions such as the aldol reaction. When considering the use of an enzyme encoded by one of the ORFs of the present invention for biotransformation and organic synthesis it is sometimes necessary to consider the respective advantages and disadvantages of using a microorganism as opposed to an isolated enzyme. Pros and cons of using a whole cell system on the one hand or an isolated partially purified enzyme on the other hand, has been described in detail by Bud *et al.*, *Chemistry in Britain* (1987), p. 127.

Amino transferases, enzymes involved in the biosynthesis and metabolism of amino acids, are useful in the catalytic production of amino acids. The advantages of using microbial based enzyme systems is that the amino transferase enzymes catalyze the stereo-selective synthesis of only *l*-amino acids and generally possess uniformly high catalytic rates. A description of the use of amino transferases for amino acid production is provided by Roselle-David, *Methods of Enzymology 136*:479 (1987).

2. Generation of Antibodies

As described here, the proteins of the present invention, as well as homologs thereof, can be used in a variety procedures and methods known in the art which are currently applied to other proteins. The proteins of the present invention can further be used to generate an antibody which selectively binds the protein. Such antibodies can be either monoclonal or polyclonal antibodies, as well fragments of these antibodies, and humanized forms.

The invention further provides antibodies which selectively bind to one of the proteins of the present invention and hybridomas which produce these antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth *et al.*, *J. Immunol. Method 35*:1-21 (1980); Kohler and Milstein, *Nature 256*:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today 4*:72 (1983); Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985), pp. 77-96).

Any animal (mouse, rabbit, etc.) which is known to produce antibodies can be immunized with the pseudogene polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or interperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of the protein encoded by the ORF of the present invention used for immunization will vary based on the animal which is immunized, the antigenicity of the peptide and the site of injection.

The protein which is used as an immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to coupling the antigen with a heterologous protein (such as globulin or β-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al.*, *Exp. Cell Res. 175*:109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to proteins of the present invention.

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

The present invention further provides the above-described antibodies in detectably labelled form. Antibodies can be detectably labelled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.) fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labelling are well-known in the art, for example see (Sternberger, L.A. *et al., J. Histochem. Cytochem. 18*:315 (1970); Bayer, E.A. *et al., Meth. Enzym. 62*:308 (1979); Engval, E. *et al., Immunol. 109*:129 (1972); Goding, J.W. *J. Immunol. Meth. 13*:215 (1976)).

The labeled antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues in which a fragment of the *Mycoplasma genitalium* genome is expressed.

The present invention further provides the above-described antibodies immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir, D.M. *et al.*, "*Handbook of Experimental Immunology*" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby, W.D. *et al., Meth. Enzym. 34* Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro, in vivo,* and *in situ* assays as well as for immunoaffinity purification of the proteins of the present invention.

3. Diagnostic Assays and Kits

The present invention further provides methods to identify the expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using one of the DFs or antibodies of the present invention.

In detail, such methods comprise incubating a test sample with one or more of the antibodies or one or more of the DFs of the present invention and assaying for binding of the DFs or antibodies to components within the test sample.

Conditions for incubating a DF or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the DF or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the DFs or antibodies of the present invention. Examples of such assays can be found in Chard, T., *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. *et al.*, *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the DFs or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound DF or antibody.

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or DF.

Types of detection reagents include labelled nucleic acid probes, labelled secondary antibodies, or in the alternative, if the primary antibody is labelled, the enzymatic, or antibody binding reagents which are capable of reacting with the labelled antibody. One skilled in the art will readily recognize that the disclosed DFs and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

4. Screening Assay for Binding Agents

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents which bind to a protein encoded by one of the ORFs of the present invention or to one of the fragments and the *Mycoplasma* genome herein described.

In detail, said method comprises the steps of:

(a) contacting an agent with an isolated protein encoded by one of the ORFs of the present invention, or an isolated fragment of the *Mycoplasma* genome; and
(b) determining whether the agent binds to said protein or said fragment.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention.

Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, Application of Synthetic Peptides: Antisense Peptides," In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY (1992), pp. 289-307, and Kaspczak *et al.*, *Biochemistry 28*:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control.

One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids Res 6*:3073 (1979); Cooney *et al.*, *Science 241*:456 (1988); and Dervan *et al.*, *Science 251*: 1360 (1991)) or to the mRNA itself (antisense - Okano, *J. Neurochem. 56*:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents which bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent, in the control of bacterial infection by modulating the activity of the protein encoded by the ORF. Agents which bind to a protein encoded by one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition for use in controlling *Mycoplasma* growth and infection.

5. Vaccine and Pharmaceutical Composition

The present invention further provides pharmaceutical agents which can be used to modulate the growth of *Mycoplasma genitalium*, or another related organism, *in vivo* or *in vitro*. As used herein, a "pharmaceutical agent" is defined as a composition of matter which can be formulated using known techniques to provide a pharmaceutical compositions. As used herein, the "pharmaceutical agents of the present invention" refers the pharmaceutical agents which are derived from the proteins encoded by the ORFs of the present invention or are agents which are identified using the herein described assays.

As used herein, a pharmaceutical agent is said to "modulated the growth of *Mycoplasma sp.*, or a related organism, *in vivo* or *in vitro*," when the agent reduces the rate of growth, rate of division, or viability of the organism in question. The pharmaceutical agents of the present invention can modulate the growth of an organism in many fashions, although an understanding of the underlying mechanism of action is not needed to practice the use of the pharmaceutical agents of the present invention. Some agents will modulate the growth by binding to an important protein thus blocking the biological activity of the protein, while other agents may bind to a component of the outer surface of the organism blocking attachment or rendering the organism more prone to act the bodies nature immune system. Alternatively, the agent may be comprise a protein encoded by one of the ORFs of the present invention and serve as a vaccine. The development and use of a vaccine based on outer membrane components, such as the LPS, are well known in the art.

As used herein, a "related organism" is a broad term which refers to any organism whose growth can be modulated by one of the pharmaceutical agents of the present invention. In general, such an organism will contain a homolog of the protein which is the target of the pharmaceutical agent or the protein used as a vaccine. As such, related organism do not need to be bacterial but may be fungal or viral pathogens.

The pharmaceutical agents and compositions of the present invention may be administered in a convenient manner such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, they are administered in an amount of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The agents of the present invention can be used in native form or can be modified to form a chemical derivative. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980).

For example, a change in the immunological character of the functional derivative, such as affinity for a given antibody, is measured by a competitive type immunoassay. Changes in immunomodulation activity are measured by the appropriate assay. Modifications of such protein properties as redox or thermal stability, biological half-life, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

The therapeutic effects of the agents of the present invention may be obtained by providing the agent to a patient by any suitable means (i.e., inhalation, intravenously, intramuscularly, subcutaneously, enterally, or parenterally). It is preferred to administer the agent of the present invention so as to achieve an effective concentration within the blood or tissue in which the growth of the organism is to be controlled.

To achieve an effective blood concentration, the preferred method is to administer the agent by injection. The administration may be by continuous infusion, or by single or multiple injections.

In providing a patient with one of the agents of the present invention, the dosage of the administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc. In general, it is desirable to provide the recipient with a dosage of agent which is in the range of from about 1 pg/kg to 10 mg/kg (body weight of patient), although a lower or higher dosage may be administered. The therapeutically effective dose can be lowered by using combinations of the agents of the present invention or another agent.

As used herein, two or more compounds or agents are said to be administered "in combination" with each other when either (1) the physiological effects of each compound, or (2) the serum concentrations of each compound can be measured at the same time. The composition of the present invention can be administered concurrently with, prior to, or following the administration of the other agent.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to decrease the rate of growth (as defined above) of the target organism.

The administration of the agent(s) of the invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the agent(s) are provided in advance of any symptoms indicative of the organisms growth. The prophylactic administration of the agent(s) serves to prevent, attenuate, or decrease the rate of onset of any subsequent infection. When provided therapeutically, the agent(s) are provided at (or shortly after) the onset of an indication of infection. The therapeutic administration of the compound(s) serves to attenuate the pathological symptoms of the infection and to increase the rate of recovery.

The agents of the present invention are administered to the mammal in a pharmaceutically acceptable form and in a therapeutically effective concentration. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

The agents of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the agents of the present invention, together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb one or more of the agents of the present invention. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate agents of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* (1980).

The invention further provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the agents of the present invention may be employed in conjunction with other therapeutic compounds.

Experimental

Example 1

Overview of Experimental Design and Methods

1. Shotgun Sequencing Strategy

The overall strategy for a shotgun approach to whole genome sequencing is outlined in Table 3. The theory of shotgun sequencing follows from the application of the equation for the Poisson distribution $p_x = m^x e/x!$, where x is the number of occurrences of an event and m is the mean number of occurrences. To determine the probability that any given base is not sequenced after a certain amount of random sequence has been generated, if L is the genome length, n is the number of clone insert ends sequenced, and w is the sequencing read length, then $m = nw/L$, and the probability that no clone originates at any of the w bases preceding a given base, i.e., the probability that the base is not sequenced, id $p_0 = e^{-m}$. Using the fold coverage as the unit form, one sees that after 580 kb of sequence has been randomly generated, m = 1, representing 1X coverage. In this case, $p_0 = e^{-1} = 37$, thus approximately 37% is unsequenced. A 5X coverage (approximately 3150 clones sequenced from both insert ends) yields $p_0 = e^{-5} = 0.0067$, or .67% unsequenced. The total gap length is Le^{-m}, and the average gap size is L/n. 5X coverage would leave about 48 gaps averaging about 80 bp in size. The treatment is essentially that of Lander and Waterman. Table 4 illustrates a computer simulation of a random sequencing experiment for coverage of a 580 kb genome with an average fragment size of 400 bp.

2. Random Library Construction

In order to approximate the random model described above during actual sequencing, a nearly ideal library of cloned genomic fragment is required. *M. genitalium* genomic chromosomal DNA was mechanically sheared, digested with BAL31 nuclease to produce blunt-ends, and size-fractionated by agarose gel electrophoresis. Fragments in the 2.0 kb size range were excised and recovered. These fragments were ligated to SmaI-cut, phophatased pUC18 vector and the ligated products were fractionated on an agarose gel. The linear vector plus insert band was excised and recovered. The ends of the linear recombinant molecules were repaired with T4 polymerase treatment and the molecules were then ligated into circles. This two-stage procedure resulted in a molecularly random collection of single-insert plasmid recombinants with minimal contamination from double-insert plasmid recombinants with minimal contamination from double-insert chimeras (<1%) or free vector (<1%). Deviation from randomness is most likely to occur during cloning. *E. coli* host cells deficient in all recombinant and restriction functions were used to prevent rearrangements, deletions, and loss of clones by restriction. Transformed cells were plated directly on antibiotic diffusion plates to avoid the usual broth recovery phase which allows multiplication and selection of the most rapidly growing cells. All colonies were picked for template preparation regardless of size. Only clones lost due to "poison" DNA or deleterious gene products would be deleted from the library, resulting in a slight increase in gap number over that expected.

In order to evaluate the quality of the *M. genitalium* random insert library, sequence data was obtained from approximately 2000 templates using the M13F primer. The random sequence fragments were assembled using The Institute for Genomic Research (TIGR) autoassembler software after obtaining 500, 1000, 1500, and 2000 sequence fragments, and the number of unique assembled base pairs was determined. The progression of assembly was plotted using the actual data obtained from the assembly of up to 2000 sequence fragments and compared the data that is provided in the ideal plot. There was essentially no deviation of the actual assembly data from the ideal plot, indicating that we had constructed close to an ideal random library with minimal contamination from double insert chimeras and free of vector.

3. Random DNA Sequencing

Five-thousand seven hundred and sixty (5,760) plasmid templates were prepared using a "boiler bead" preparation method developed in collaboration with AGTC (Gaithersburg, MD), as suggested by the manufacturer. The AGTC method is performed in a 96-well format for all stages of DNA preparation from bacterial growth through final DNA purification. Template concentration was determined using Hoechst Dye and a Millipore Cytofluor. DNA concentrations were not adjusted and low-yielding templates were identified and not sequenced where possible. Sequencing reactions were carried out on plasmid templates using the AB Catalyst Lab station or Perkin-Elmer 9600 Thermocyclers with Applied Biosystems PRISM Ready Reaction Dye Primer Cycle Sequencing Kits for the M13 forward (-21M13) and the M13 reverse (RP1) primers. Dye terminator sequencing reactions were carried out on the lambda templates on a Perkin-Elmer 9600 Thermocyler using the Applied Biosystems Ready Reaction Dye Terminator Cycle Sequencing kits. Nine-thousand eight hundred and forty-six (9,846) sequencing reactions were performed during the random phase of the project by 4 individuals using an average of 10 AB373 DNA Sequencers over a 2 month period. All sequencing reactions were analyzed using the Stretch modification of the AB373, primarily using a 36cm well-to-read distance. The overall sequencing success rate for M13-21 sequences was 88% and 84% for M13RP1 sequences. The average usable read length for M13-21 sequences was 485 and 441 for M13RP1 sequences.

The art has described the value of using sequence from both ends of sequencing templates to facilitate ordering of contigs in shotgun assembly projects. A skilled artisan must balance the desirability of both-end sequencing (including the reduced cost of lower total number of templates) against shorter read-lengths and lower success rates for sequencing reactions performed with the M13RP1 (reverse) primer compared to the M13-21 (forward) primer. For this project, essentially all of the templates were sequenced from both ends.

4. Protocol for Automated Cycle Sequencing

The sequencing consisted of using five (5) ABI Catalyst robots and ten (10) ABI 373 Automated DNA Sequencers. The Catalyst robot is a publicly available sophisticated pipetting and temperature control robot which has been developed specifically for DNA sequencing reactions. The Catalyst combines pre-aliquoted templates and reaction mixes consisting of deoxy- and dideoxynucleotides, the *Taq* thermostable DNA polymerase, fluorescently-labelled sequencing primers, and reaction buffer. Reaction mixes and templates were combined in the wells of an aluminum 96-well thermocycling plate. Thirty consecutive cycles of linear amplification (e.g., one primer synthesis) steps were performed including denaturation, annealing of primer and template, and extension of DNA synthesis. A heated lid with rubber gaskets on the thermocycling plate prevented evaporation without the need for an oil overlay.

Two sequencing protocols were used: dye-labelled primers and dye-labelled dideoxy chain terminators. The shotgun sequencing involves use of four dye-labelled sequencing primers, one for each of the four terminator nucleotide. Each dye-primer is labelled with a different fluorescent dye, permitting the four individual reactions to be combined into one lane of the 373 DNA Sequencer for electrophoresis, detection, and base-calling. ABI currently supplies pre-mixed reaction mixes in bulk packages containing all the necessary non-template reagents for sequencing. Sequencing can be done with both plasmid and PCR-generated templates with both dye-primers and dye-terminators with approximately equal fidelity, although plasmid templates generally give longer usable sequences.

Thirty-two reactions were loaded per 373 Sequencer each day, for a total of 960 samples. Electrophoresis was run overnight following the manufacture's protocols, and the data was collected for twelve hours. Following electrophoresis and fluorescence detection, the ABI 373 performs automatic lane tracking and base-calling. The lane-tracking was confirmed visually. Each sequence electropherogram (or fluorescence lane trace) was inspected visually and assessed for quality. Trailing sequences of low quality were removed and the sequence itself was loaded via software a Sybase database (archived daily to a 8mm tape). Leading vector polylinker sequence was removed automatically by software program. The average edited lengths of sequences from the ABI 373 Sequencers converted to Stretch Liners were approximately 460 bp.

Informatics

1. Data Management

A number of information management systems (LIMS) for a large-scale sequencing lab have been developed. A system was used which allowed an automated data flow wherever possible to reduce user error. The system used to collect and assemble the sequence information obtained is centered upon a relational data management system built using the Sybase RDBMS. The database is designed to store and correlate all information collected during the entire operation from template preparation to final analysis of the genome. Because the raw output of the AB 373 Sequencers is based on a Macintosh platform and the data management system chosen is based on a Unix platform, it was necessary to design and implement a variety of multi-user, client server applications which allow the raw data as well as analysis results to flow seamlessly into the database with a minimum of user effort.

2. Assembly

The sequence data from 8,472 sequence fragments was used to assemble the *M. genitalium* genome. The assembly was performed by using a new assembly engine (TIGR Assembler - previously designated ASMG) developed at TIGR. The TIGR Assembler simultaneously clusters and assembles fragments of the genome. In order to obtain the necessary speed, the TIGR Assembler builds a hash table of 10bp oligonucleotide subsequences to generate a list of potential sequence fragment. The number of potential overlaps for each fragment determines which fragments are likely to fall into repetitive elements. Beginning with a single seed sequence fragment, the TIGR Assembler extends the current contig by attempting to add the best matching fragment based on oligonucleotide content. The current contig and candidate fragment are aligned using a modified version of the Smith-Waterman algorithm which provides for optimal gap alignments. The current contig is extended by the fragment only if strict criteria for the quality of the match are met. The match criteria include the minimum length of overlap, the maximum length of an unmatched end, and the minimum percentage match. These criteria are automatically lowered by the TIGR Assembler in regions of minimal coverage and raised in regions with a good chance of containing repetitive elements. Potentially chimeric fragments and fragments representing the boundaries of repetitive elements are often rejected based on partial mismatches at the ends of alignments and excluded from the current contig. The TIGR Assembler is designed to take advantage of clone size information coupled with sequencing from both ends of each template. The TIGR Assembler enforces the constraint that sequence fragments from two ends of the same template point toward one another in the contig and are located within a certain range of base pairs (definable for each clone based on the known clone size range for a given library). Assembly of the 8,472 sequence fragments of *M. genitalium* required 10 hours of CPU time on a SPARCenter 2000. All contigs were loaded into a Sybase structure representing the location of each fragment in the contig and extensive information about the consensus sequence itself. The result of this process was approximately 40 contigs ordered into 2 groups (See below). Because of the high stringency of the TIGR Assembler process it was found to be useful to perform a FASTA (GRASTA) alignment of all contigs built by the TIGR Assembler process against each other. In this way additional overlaps were detected which enabled compression of the data set into 26 contigs in 2 groups.

Achieving Closure

The complete genome sequence was obtained by sequencing across the gaps between contigs. While gap filling has occupied a major portion of the time and expense of other genome sequencing projects, it was minimal in the present invention. This was primarily due to 1) saturation of the genome as a result of the number of random clones and sequencing reactions performed, 2) the longer read lengths obtained from the Stretch Liners, 3) the anchored ends which were obtain for joining contigs, and 4) the overall capacity and efficiency of the high throughput sequencing facility.

Gaps occurred on a predicted random basis, as shown in Table 4, which illustrates simulated random sequencing. These gaps generally were less than 200 bp in size. All of the gaps were closed by sequencing further on the templates bordering the gaps. In these cases, oligo primers for extension of the sequence from both ends of the gap were generated using techniques known in the art. This gave a double standard coverage across the gap areas.

The high redundancy of sequence information that was obtained from the shotgun approach gave a highly accurate sequence. Our sequence accuracy was confirmed by comparing the sequence information obtained against known *M. genitalium* genes present in the GenBank database. The accuracy of our chromosome structure was confirmed by comparison of restriction digests to the known restriction map of *M. genitalium*. The EcoRI restriction map of *M. genitalium* is shown in Figure 1 and expressed in tabular form in Table 5.

Identifying Genes

M. genitalium ORFs were initially defined by evaluating their coding potential with the program Gene Works using composition matrices specific to *Mycoplasma* genomic DNA. The ORF sequences (plus 300 bp of flanking sequence) were used in searches against a database of non-redundant bacterial proteins (NRBP). Redundancy was removed from NRBP at two stages. (1) All DNA coding sequences were extracted from GenBank (release 85), and sequences from the same species were searched against each other. Sequences having >97% similarity over regions >100 nucleotides were combined. (2) The sequences were translated and used to protein comparisons with all sequences in Swiss-Prot (release 30). Sequences belonging to the same species and having >98% similarity over 33 amino acids were combined. NRBP is composed of 21445 sequences from 23751 GenBank sequences and 11183 Swiss-Prot sequences from 1099 different species.

Searches were performed using an algorithm that (1) translates the query DNA sequence in all six reading frames for searching against a protein database, (2) identifies the protein sequences that match the query, and (3) aligns the protein-protein matches using a modified Smith-Waterman algorithm. In cases where insertion or deletions in the DNA sequence produced a frame shift error, the alignment algorithm started with protein regions of maximum similarity and extended the alignment to the same database match using the 300 bp flanking region. Regions known to contain frame shift errors were saved to the database and evaluated for possible correction. The role categories were adopted from those previously defined by Riley *et al.* for *E. coli* gene products. Role assignments were made to *M. genitalium* ORFS at the protein sequence level by linking the protein sequence of the ORFS with the Swiss-Prot sequences in the Riley database.

Detailed Description of Sequencing the Mycoplasma genitalium Genome, Genome Analysis and Comparative Genomics

We have determined the complete nucleotide sequence (580,071 bp) of the *Mycoplasma genitalium* genome using the approach of whole chromosome shotgun sequencing and assembly, which has successfully been applied to the analysis of the *Haemophilus influenzae* genome (R. Fleischmann *et al., Science 269*:496 (1995)). These data, together with the description of the complete genome sequence (1.83 Mb) of the eubacterium *Haemophilus influenzae*, have provided the opportunity for comparative genomics on a whole genome level for the first time. Our initial whole genome comparisons reveal fundamental differences in genome content which are reflected in different physiological ad metabolic capacities of *M. genitalium* and *H. influenzae*.

The strategy and methodology for whole genome shotgun sequencing id assembly was similar to that previously described for *H. influenzae* (R. Fleischmann *et al., Science 269*:496 (1995). In particular, a total of 50 μg of purified *M. genitalium* strain G-37 DNA (ATCC No. 33530) was isolated from cells grown in Hayflick's medium. A mixture (990 μl) containing 50 μg of DNA, 300 mM sodium acetate, 10 mM tris HCl, 1 mM EDTA, and 30 percent glycerol was chilled to O°C in a nebulizer chamber and sheared at 4 lbs/in^2 for 60 seconds. The DNA was precipitated in ethanol and redissolved in 50 μl of tris-EDTA (TE) buffer to create blunt ends; a 40 μl portion was digested for 10 minutes at 30°C in 85 μl of BAL31 buffer with 2 units of BAL 31 nuclease (New England BioLabs). The DNA was extracted with phenol, precipitated in ethanol, dissolved in 60 μl of TE buffer, and fractionated on a 1.0 percent low melting agarose gel. A fraction (2.0 kb) was excised, extracted with phenol, and redissolved in 20 μl of TE buffer. A two-step ligation procedure was used to produce a plasmid library in which 99% oft he recombinants contained inserts, of which >99% were single inserts. The first ligation mixture (50 μl) contained approximately 2 μg DNA fragments, 2 μg of SmaI + bacterial alkaline phosphatase pUC 18 DNA (Pharmacia), and 10 units of T4 DNA hgase (GIBCO/BRL), and incubation was for 5 hours at 4°C. After extraction with phenol and ethanol precipitation, the DNA was dissolved in 20 μl of TE buffer and separated by electrophoresis on a 1.0 percent low melting agarose gel. A ladder of ethidium bromide-stained, linearized DNA bands, identified by size as insert (i), vector (v), v + i, v + 2i, v + 3i, etc. was visualized by 360 nm ultraviolet light. The v + i DNA was excised and recovered in 20μl of TE buffer. The v + i DNA was blunt-ended by T4 polymerase treatment for 5 minutes at 37°C in a reaction mixture (50μl) containing the linerized v + i fragments, four deoxynucleotide triphosphates (dNTPs) (25 μM each), and 3 units of T4 polymerase (New England Biolabs) under buffer conditions recommended by the supplier. After phenol extraction and ethanol precipitation, the repaired v + i linear pieces were dissolved in 20 μl of TE. The final ligation to produce circles was carried out in a 50 μl reaction containing 5 μl of v + i DNA and 5 units of T4 hgase at 15°C overnight. The reaction mixture was heated at 67°C for 10 minutes and stored at -20°C.

For transformation, a 100 μl portion of Epicurian SURE 2 Supercompetent Cells (Stratagene 200152) was thawed on ice and transferred to a chilled Falcon 2059 tube on ice. A 1.7 μl volume of 1.42M β-mercaptoethanol was added to the cells to a final concentration of 25 mM. Cells were incubated on ice for 10 minutes. A 1 μl sample of the final ligation mix was added to the cells and incubated on ice for 30 minutes. The cells were heat-treated for 30 seconds at 42°C and placed back on ice for 2 minutes. The outgrowth period in liquid culture was omitted to minimize the preferential growth of any transformed cell. Instead, the transformed cells were plated directly on a nutrient rich SOB plate containing a 5 ml bottom layer of SOB agar (1.5 percent SOB agar consisted of 20 g of tryptone, 5g of yeast extract, 0.5 g of NaCl, and 1.5 percent Difco agar/liter). The 5 ml bottom layer was supplemented with 0.4 ml of ampicillin (50 mg/ml) per 100 ml of SOB agar. The 15 ml top layer of SOB agar was supplemented with 1 ml of $MgCl_2$ (1M) and 1 ml of $MgSO_4$ (1M) per 100 ml of SOB agar. The 15 ml top layer was poured just before plating. The titer of the library was approximately 100 colonies per 10 μl aliquot of transformation.

One of the lessons learned from sequencing and assembly of the complete *H. influenzae* genome was that contig ordering and gap closure is most efficient if the random sequencing phase of the project is continued until at least 99.8%-99.9% of the genome is sequenced with at least 6-fold coverage. To calculate the number of random sequencing reactions necessary to obtain this coverage for the *M. genitalium* genome, we made use of the Lander and Waterman [E.S. Lander and M.S. Waterman, *Genomics 2*:231 (1988)] application of the Poisson distribution, where $p_x = e^{-nw/L}$. p_x is the probability that any given base is not sequenced, n is the number of clone insert ends sequenced, w is the average read length of each template in bp, and L is the size of the genome in bp. For a genome of 580 kb with an average sequencing read length of 450 bp after editing, approximately 8650 sequencing reactions (or 4325 clones sequenced from both ends) should theoretically provide 99.85% coverage of the genome. This level of coverage should leave approximately 10 gaps with an average size of 70 bp unsequenced.

To evaluate the quality of the *M. genitalium* library, sequence data were obtained from both ends of approximately 600 templates using both the M13 forward (M13-21) and the M13 reverse (M13RP1) primers. Sequence fragments were assembled using the TIGR ASSEMBLER and found to approximate a Poisson distribution of fragments with an average read length of 450 bp for a 580 kb library, indicating that the library was essentially random.

For this project, a total of 5760 double-stranded DNA plasmid templates were prepared in a 96-well format using a boiling bead method. Ninety-four percent of the templates prepared yielded a DNA concentration ≥ 30 ng/μl and were used for sequencing reactions. To facilitate ordering of contigs each template was sequenced from both ends. Reactions were carried out on using the AB Catalyst LabStation with Applied Biosystems PRISM Ready reaction Dye Primer Cycle Sequencing Kits for the M13 forward (M13-21) and the M13 reverse (M13RP1) primers. The success rate and average read length after editing with the M13-21 primer were 88 percent and 444 bp, respectively, and 84 percent and 435 bp, respectively, with the M13RP1 primer. All data from template preparation to final analysis of the project were stored in a relational data management system developed at TIGR [A.R. Kerlavage *et al.*, *Proceedings of the Twenty-Sixth Annual Hawaii International Conference on System Science* (IEEE Computer Society Press, Washington, D.C., 1993), p. 585] To facilitate ordering of contigs each template was sequenced from both ends. A total of 9846 sequencing reactions were performed by five individuals using an average of 8 AB 373 DNA Sequencers per day for a total of 8 weeks. Assembly of 8472 high quality *M. genitalium* sequence fragments along with 299 random genomic sequences from Peterson *et al.* (S.N. Peterson *et al.*, *J. Bacteriol. 175*:7918 (1993)) was performed with the TIGR ASSEMBLER. The assembly process generated 39 contigs (size range: 606 to 73,351 bp) which contained a total of 3,806,280 bp of primary DNA sequence data. Contigs were ordered by ASM_ALIGN, program which links contigs based on information derived from forward and reverse sequencing reactions from the same clone.

ASM_ALIGN analysis revealed that all 39 gaps were spanned by an existing template from the small insert genomic DNA library (i.e., there were no physical gaps in the sequence assembly). The order of the contigs was confirmed by comparing the order of the random genomic sequences from Peterson *et al.* (S.N. Peterson *et al.*, *J. Bacteriol. 175*:7918 (1993)) that were incorporate into the assembly with their known position on the physical map of the *M. genitalium* chromosome (T.S. Lucier *et al.*, *Gene 150*:27 (1994); Peterson *et al.*, *J. Bacteriol. 177*:3199 (1995)). Because of the high stringency of the TIGR ASSEMBLER, the 39 contigs were searched against each other with GRASTA (a modified FASTA (B. Brutlag *et al.*, *Comp. Chem. 1*:203 (1993)). The BLOSUM 60 amino acid substitution matrix was used in all protein-protein comparisons [S. Henikoff and J.G. Henikoff, *Proc. Natl. Acad. Sci. USA 89*:1091 (1992)] to detect overlaps (< 30 bp) that would have been missed during the initial assembly process. Eleven overlaps were detected with this approach which reduced the total number of gaps from 39 to 28.

Templates spanning each of the sequence gaps were identified and oligonucleotide primers were designed from the sequences at the end of each contig. All gaps were less than 300 bp; thus a primer walk from both ends of each template was sufficient for closure. All electropherograms were visually inspected with TIGR EDITOR (R. Fleischmann *et al.*, *Science 269*:496 (1995)) for initial sequence editing. Where a discrepancy could not be resolved o a clear assignment made, the automatic base calls were left unchanged.

Several criteria for determination of sequence completion were established for the *H. influenzae* genome sequencing project ad these same criteria were applied to this study. Across the assembled *M. genitalium* genome there is an average sequence redundancy of 6.5-fold. The completed sequence contains less than 1% single sequence coverage. For each of the 53 ambiguities remaining after editing and the 25 potential frameshifts found after sequence-similarity searching, the appropriate template was resequenced with an alternative sequencing chemistry (dye terminator vs. dye primer) to resolve ambiguities. Although it is extremely difficult to assess sequence accuracy, we estimate our error rate to be less than 1 base in 10,000 based upon frequency of shifts in open reading frames, unresolved ambiguities, overall quality of raw data, and fold coverage.

A direct cost estimate for sequencing, assembly, and annotation of the *M. genitalium* genome was determined by summing reagent and labor costs for library construction, template preparation and sequencing, gap closure, sequence confirmation, annotation, and preparation for publication, and dividing by the size of the genome in base pairs. This yielded a final cost of 30 cents per finished base pair.

Genomic Analysis

The *M. genitalium* genome is a circular chromosome of 580,071 bp. The overall G+C content is 32% (A, 34%; C, 16%; G, 16%; and T, 34%). The G+C content across the genome varies between 27 and 37% (using a window of 5000 bp), with the regions of lowest G+C content flanking the presumed origin of replication of the organism. As in *H. influenzae* (Fleischmann, R. *et al., Science 269*:496 (1995)), the rRNA operon in *M. genitalium* contains a higher G+C content (44%) than the rest of the genome, as do the tRNA genes (52%). The higher G+C content in these regions may reflect the necessity of retaining essential G+C base pairing for secondary structure in rRNAs and tRNAs (Rogers, M.J. *et al., Isr. J. Med. Sci. 20*:768 (1984)).

The genome of *M. genitalium* contains 74 *EcoRI* fragments, as predicted by cosmid mapping data (Lucier, T.S. *et al., Gene 150*:27 (1994); Peterson *et al., J. Bacteriol. 177*:3199 (1995)). The order and sizes of the *EcoRI* fragments determined from sequence analysis are in agreement with those previously reported (Lucier, T.S. *et al., Gene 150*:27 (1994); Peterson *et al., J. Bacteriol. 177*:3199 (1995)), with one apparent discrepancy between coordinates 62,708 and 94,573 in the sequence. However, re-evaluation of cosmid hybridization data in light of results from genome sequence analysis confirms that the sequence data are correct, and the extra 4.0 kb *EcoRI* fragment in this region of the cosmid map reflects a misinterpretation of the overlap between cosmids J-8 and 21 (Lucier, T.S., unpublished observation). The ends of each clone from the ordered cosmid library were sequenced and are shown on the circular chromosome in Figure 4. The order of the cosmids based on sequence analysis is in complete agreement with that determined by physical mapping (Lucier, T.S. *et al., Gene 150*:27 (1994); Peterson *et al., J. Bacteriol. 177*:3199 (1995)).

We defined the first bp of the chromosomal sequence of *M. genitalium* based on the putative origin of replication (Bailey & Bott, *J. Bacteriol. 176*:5814 (1994)). Studies of origins of replication in some prokaryotes have shown that DNA synthesis is initiated in an untranscribed AT rich region between *dnaA* and *dnaN* (Ogasawara, N. *et al.*, in *The Bacterial Chromosome*, Krlica & Riley, eds., American Society for Microbiology, Washington, DC (1990), pp. 287-295; Ogasawara & Yoshikawa, *Mol. Microbiol. 6*:629 (1992)). A search of the *M. genitalium* sequence for "DnaA boxes" around the putative origin of replication with consensus "DnaA boxes" from *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* revealed no significant matches. Although we have not been able to precisely localize the origin, the co-localization of dnaA and dnaN to a 4000 bp region of the chromosome lends support to the hypothesis that it is the functional origin of replication in *M. genitalium* (Ogasawara, N. *et al.*, in *The Bacterial Chromosome*, Krlica & Riley, eds., American Society for Microbiology, Washington, DC (1990), pp. 287-295; Ogasawara & Yoshikawa, *Mol. Microbiol. 6*:629 (1992), Miyata, M. *et al., Nucleic Acids Res. 21*:4816 (1993)). We have chosen an untranscribed region between *dnaA* and *dnaN* so that *dnaN* is numbered as the first open reading frame in the genome. As seen in Figure 4, genes to the right of this region are preferentially transcribed from the plus strand and to the left of this region, are preferentially transcribed from the minus strand. The apparent polarity in gene transcription is maintained across each half of the genome (Figures 4 and 5). This stands in marked contrast to *H. influenzae* which displays no apparent polarity of transcription around the origin of replication. The significance of this observation remains to be determined.

The predicted coding regions of *M. genitalium* were initially defined by searching the entire genome for open reading frames greater than 100 amino acids. Translations were made using the genetic code for mycoplasma species in which UGA encodes tryptophan. All open reading frames were searched with BLAZE (Brutlag, D. *et al., Comp. Chem. 1*:203 (1993). The BLOSUM 60 amino acid substitution matrix was used in all protein-protein comparisons (Henikoff, S. and Henikoff, J.G., *Proc. Natl. Acad. Sci. USA 89*:1091 (1992)) against a non-redundant bacterial protein database (NRBP) (Fleischmann, R. *et al., Science 269*:496 (1995)) developed at TIGR on a MasPar MP-2 massively parallel computer with 4096 microprocessors. Protein matches were aligned with PRAZE, a modified Smith-Waterman (Waterman, M.S., *Methods Enzymol. 164*:765 (1988)) algorithm. Segments between predicted coding regions of the genome were used in additional searches against all protein sequences from GenPept, Swiss-Prot, and PIR. Pairwise alignments between *M. genitalium* predicted open reading frames and sequences from the public archives were examined. Motif matches were annotated in cases where sequence similarity was confined to short domains in the predicted coding region. The coding potential of 170 unidentified open reading frames was analyzed with GeneMark (Borodovsky & McIninch, ibid, p. 123) which had been trained with 308 *M. genitalium* sequences. Open reading frames that had low coding potential (based on the GeneMark analysis) and were smaller than. 100 nucleotides (a total of 53) were removed from the final set of putative coding regions. In a separate analysis, open reading frames were searched against the complete set of translated sequences from *H. influenzae* (GSDB accession L42023, see (Fleischmann, R. *et al., Science 269*:496 (1995))). In total, these processes resulted in the identification of 482 predicted coding regions, of which 365 were putatively identified (Twenty-three of the protein matches in Table 6 were annotated as motifs. These data matches were not full-length protein matches, but nonetheless displayed regions of significant amino acid similarity) and 117 had no matches to protein sequences from any other organism.

The 365 predicted coding regions that matched protein sequences from the public sequence archives were assigned biological roles. The role classifications were developed from Riley (Riley, M., *Microbiol. Rev. 57*:862 (1992)) and identical to those used in *H. influenzae* assignments (Fleischmann, R. *et al*., *Science 269*:496 (1995)). A separate search procedure was used in cases where we were unable to detect genes in the *M. genitalium* genome. Query peptide sequences that were available from eubacteria such as *E. coli*, *B. subtilis*, *M. capricolum*, and *H. influenzae* were used in searches against all six reading frame translations of the entire genome sequence, and the alignments were examined. The possibility remains that current searching methods, an incomplete set of query sequences, or the subjective analysis of the database matches, are not sensitive enough to identify certain *M. genitalium* gene sequences.

One-half of all predicted coding regions in *M. genitalium* for which a putative identification could be assigned display the greatest degree of similarity to a protein from either a gram-positive organism (e.g., *B. subtilis*) or a *Mycoplasma* species. The significance of this finding is underscored by the fact that NRBP contained 3885 sequences from *E. coli* and only 1975 sequences from *B. subtilis*. In the majority of cases where *M. genitalium* coding regions matched sequences from both *E. coli* and *Bacillus* species, the better match was to a sequence from *Bacillus* (average of 62 percent similarity) rather than to a sequence from *E. coli* (average of 56 percent similarity). The evolutionary relationship between *Mycoplasma* and the *Lactobacillus-Clostridium* branch of the gram-positive phylum has been deduced from small subunit rRNA sequences (Maidak, B.L. *et al*., *Nucleic Acids Research 22*:3485 (1994)). Our data from whole genome analysis support this hypothesis.

Comparative Genomics: M. genitalium and H. influenzae

A survey of the genes and their organization in *M. genitalium* makes possible the description of a minimal set of genes required for survival. One would predict that a minimal cell must contain genes for replication and transcription, at least one rRNA operon and a set of ribosomal proteins, tRNAs and tRNA synthetases, transport proteins to derive nutrients from the environment, biochemical pathways to generate ATP and reducing power, and mechanisms for maintaining cellular homeostasis. Comparison of the genes identified in *M. genitalium* with those in *H. influenzae* allows for identification of a basic complement of genes conserved in these two species and provides insights into physiological differences between one of the simplest self-replicating prokaryotes and a more complex, gram-negative bacterium.

The *M. genitalium* genome contains 482 predicted coding sequences (Table 6) as compared to 1, 727 identified in *H. influenzae* (Fleischmann, R. *et al*., *Science 269*:496 (1995)). Table 7 summarizes the gene content of both organisms sorted by functional category. The percent of the total genome in *M. genitalium* and *H. influenzae* encoding genes involved in cell envelope, cellular processes, energy metabolism, purine and pyrimdine metabolism, replication, transcription, transport, and other categories is similar; although the total number of genes in these categories is considerably fewer in *M. genitalium*. A smaller percentage of the *M. genitalium* genome encodes genes involved in amino acid biosynthesis, biosynthesis of co-factors, central intermediary metabolism, fatty acid and phospholipid metabolism, and regulatory functions as compared with *H. influenzae*. A greater percentage of the *M. genitalium* genome encodes proteins involved in translation than in *H. influenzae*, as shown by the similar numbers of ribosomal proteins and tRNA synthetases 'in both organisms.

The 482 predicted coding regions in *M. genitalium* (average size of 1100 bp) cover 85% of the genome (on average, one gene every 1169 bp), a value similar to that found in *H. influenzae* where 1727 predicted coding regions (average size of 900 bp) cover 91% of the genome (one gene every 1042 bp). These data indicate that the reduction in genome size that has occurred within *Mycoplasma* has not led to an increase in gene density or a decrease in gene size (Bork, P. *et al*., *Mol. Microbiol. 16*:955 (1995)). A global search of *M. genitalium* and *H. influenzae* genomes reveals short regions of conservation of gene order, particularly two clusters of ribosomal proteins.

Replication. Two major protein complexes are formed during replication: the primosome and the replisome. We have identified genes encoding many of the essential proteins in the replication process, including *M. genitalium* isologs of the primosome proteins DnaA, DnaB, GyrA, GyrB, a single stranded DNA binding protein, and the primase protein, DnaE. DnaJ and DnaK, heat shock proteins that may function in the release of the primosome complex, are also found in *M. genitalium*. A gene encoding the DnaC protein, responsible for delivery of DnaB to the primosome, has yet to be identified.

Genes encoding most of the essential subunit proteins for DNA polymerase III in *M. genitalium* were also identified. The *polC* gene encodes the α subunit which contains the polymerase activity. We have also identified the isolog of *dnaH* in *B. subtilis* (dnaX in *E. coli*) which encodes the γ and t subunits as alternative products from the same gene. These proteins are necessary for the processivity of DNA polymerase III. An isolog of *dnaN* which encodes the β subunit was previously identified in *M. genitalium* (Bailey & Bott, *J. Bacteriol. 176*:5814 (1994)) and is involved in the process of clamping the polymerase to the DNA template. While we have yet to identify a gene encoding the ε subunit responsible for the 3'-5' proofreading activity, it is possible that this activity is encoded in the α subunit as has been previously described (Sanjanwala, B. and Ganesa, A.T., *Mol. Gen. Genet. 226*:467 (1991); Sanjanwala, B. and Ganesan, A.T., *Proc. Natl. Acad. Sci. USA 86*:4421 (1989)). Finally, we have identified a gene encoding a DNA ligase, necessary for the joining of the Okazaki fragments formed during synthesis of the lagging strand.

While we have identified genes encoding many of the isologs thought to be essential for DNA replication, some genes encoding proteins with key functions have yet to be identified. Examples of these are the DnaC protein mentioned above as well as Dnaθ and Dnaδ whose functions are less well understood but are thought to be involved in the assembly and processivity of polymerase III. Also apparently absent is a specific RNaseH protein responsible for the hydrolysis of the RNA primer synthesized during lagging strand synthesis.

DNA Repair. It has been suggested that in *E. coli* as may as 100 genes are involved in DNA repair (Kornberg, A. and Baker, T.A., *DNA Replication-2nd Ed.*, W.H. Freeman and Co., New York (1992)), and in *H. influenzae* the number of putatively identified DNA repair enzymes is approximately 30 (Fleischmann, R. *et al.*, *Science 269*:496 (1995)). Although *M. genitalium* appears to have the necessary genes to repair many of the more common lesions in DNA, the number of genes devoted to the task is much smaller. Excision repair of regions containing missing bases (apurinic/apyriminic (AP) sites) can likely occur by a pathway involving endonuclease IV (info), Pol I, and ligase. The *ung* gene which encodes uracil-DNA glycosylase is present. This activity removes uracil residues from DNA which usually arise by spontaneous deamination of cytosine. This produces an AP site which could then be repaired as described above.

All three genes necessary for production of the uvr ABC exinuclease are present, and along with Pol I, helicase II, and ligase should provide a mechanism for repair of damage such as cross-linking, which requires replacement of both strands. Although *recA* is present, which in *E. coli* is activated as it binds to single strand DNA, thereby initiating the SOS response, we find no evidence for a *lexA* gene which encodes the repressor which regulates the SOS genes. We have not identified photolyase (*phr*) in *M. genitalium* which repairs UV-induced pyrimidine dimers, or other genes involved in reversal of DNA damage rather than excision and replacement of the lesion.

Transcription. The critical components for transcription were identified in *M. genitalium*. In addition to the a, b, and b-prime subunits of the core RNA polymerase, *M. genitalium* appears to encode a single a factor, whereas *E. coli* and *B. subtilis* encode at least six and seven, respectively. We have not detected a homolog of the Rho termination factor gene, so it seems likely that a mechanism similar to Rho-independent termination in *E. coli* operates in *M. genitalium*. We have clear evidence for homologs of only two other genes which modulate transcription, *nusA* and *nusG*.

Translation. *M. genitalium* possesses a single rRNA operon which contains three rRNA subunits in the order: 16S rRNA(1518 bp)-spacer (203 bp)-23S rRNA (2905 bp)-spacer (56 bp)-5S rRNA (103 bp). The small subunit rRNA sequence was compared with the Ribosomal Database Project's (Maidak, B.L. *et al.*, *Nucleic Acids Research 22*:3485 (1994)) prokaryote database with the program "similarity_yank." Our sequence is identical to the *M. genitalium* (strain G37) sequence deposited there, and the 10 most similar taxa returned by this search are also in the genus *Mycoplasma*.

A total of 33 tRNA genes were identified in *M. genitalium*, these were organized into five clusters plus nine single genes. In all cases, the best match for each tRNA gene in *M. genitalium* was the corresponding gene in *M. pneumoniae* (Simoneau, P. *et al.*, *Nuc. Acid Res. 21*:4967 (1993)). Furthermore, the grouping of tRNAs into clusters (trnA, trnB, trnC, trnD, and trnE) was identical in *M. genitalium* and *M. pneumoniae* as was gene order within the cluster (Simoneau, P. *et al.*, *Nuc. Acids Res. 21*:4967 (1993)). The only difference between *M. genitalium* and *M. pneumoniae* observed with regard to tRNA gene organization was an inversion between trnD and GTG. In contrast to *H. influenzae* and many other eubacteria, no tRNAs were found 'in the spacer region between the 16S and 23S rRNA genes in the rRNA operon of *M. genitalium*, similar to what has been reported for *M. capricolum* (Sawada, M. *et al.*, *Mol. Gen. Genet. 182*:502 (1981)).

A search of the *M. genitalium* genome for tRNA synthetase genes identified all of the expected genes with the exception of glutaminyl tRNA synthetase. We expect that this gene is present in the *M. genitalium* genome, but we have not been able to identify it by similarity searches. The latest GenBank release (release 89) contains only a single entry for a glutaminyl tRNA synthetase from a bacterial species; this was from *E. coli*, a gram-negative organism only distantly related to *Mycoplasma*. In general, tRNA synthetase sequences from gram-positive organisms such as *B. subtilis* displayed greater similarity to those from *M. genitalium* than the corresponding sequences from *E. coli*, lending support to the notion that the similarity between the *E. coli* and *M. genitalium* glutaminyl tRNA synthetase may not have been high enough to be detected.

Metabolic pathways. The reduction in genome size among *Mycoplasma* species is associated with a marked reduction in the number and components of biosynthetic pathways in these organisms, requiring them to use metabolic products from their hosts. In the laboratory, *M. genitalium* has not been grown in a chemically defined medium. The complex growth requirements of this organism can be explained by the almost complete lack of enzymes involved in amino acid biosynthesis, *de novo* nucleotide biosynthesis, and fatty acid biosynthesis (Table 6 and Figure 5). When the number of genes in the categories of central intermediary metabolism, energy metabolism, and fatty acid and phospholipid metabolism are summed, marked differences in gene content between *H. influenzae* and *M. genitalium* are apparent. For example, whereas the *H. influenzae* genome contains 68 genes involved in amino acid biosynthesis, the *M. genitalium* genome contains only one. In total, the *H. influenzae* genome has 167 genes associated with metabolic pathways whereas the *M. genitalium* genome has just 42. A recent analysis of 214 kb of sequence from *Mycoplasma capricolum* (Bork, P. *et al.*, *Mol. Microbiol. 16*:955 (1995)), a related organism whose genome size is twice as large as that of *M. genitalium*, reveals that M. *capricolum* contains a number of biosynthetic enzymes not present in *M. genitalium*. This observation suggests that *M. capricolum*'s larger genome confers a greater anabolic capacity.

M. genitalium is a facultative anaerobe that ferments glucose and possibly other sugars via glycolysis to lactate and acetate. Genes that encode all the enzymes of the glycolytic pathway were identified, including genes for components of the pyruvate dehydrogenase complex, phosphotransacetylase, ad acetate kinase. The major route for ATP synthesis may be through substrate level phosphorylation since no cytochromes are present. *M. genitalium* also lacks all the components of the tricarboxylic acid cycle. None of the genes coding for glycogen or poly-beta-hydroxybutryate production were identified, indicating limited capacity for carbon and energy storage. The pentose phosphate pathway also appears limited since only genes encoding 6-phosphogluconate dehydrogenase and transketolase were identified. The limited metabolic capacity of *M. genitalium* sharply contrasts with the complexity of catabolic pathways in *H. influenzae*, reflecting the four-fold greater number of genes involved in energy metabolism found in *H. influenzae*.

Transport. The transporters identified in *H. influenzae* are specific for a range of nutritional substrates. Using protein transport as an example, both oligopeptide and amino acid transporters are represented. One interesting peptide transporter has homology to a lactococcin transporter (lcnDR3) and related bacteriocin transporters, suggesting the *M. genitalium* may export a small peptide with antibacterial activity. The *H. influenzae* isolog of the *M. hyorhinis* p37 high-affinity transport system also has a conserved lipid modification site, providing further evidence that the *Mycoplasma* binding-protein dependent transport systems are organized in a manner analogous to gram positive bacteria (Gilson, E. *et al., EMBO J. 7*:3971 (1988)).

Genes encoding proteins that function in the transport of glucose via the phosphoenolpyruvate:sugar transferase system (PTS) have been identified in *M. genitalium*. These include enzyme I (EI), HPr and sugar specific enzyme IIs (EII) (Postma, P.W. *et al., Microbiol. Rev. 57*:543 (1993)). EIIs consist of a complex of at least there domains, EIIA, EIIB and EIIC. In some bacteria (eg, *E. coli*), EIIA is a soluble protein, while in others (*Bacillus subtilis*), a single membrane protein contains all three domains, EIIA, B and C. These variations in the proteins that make up the EII complex are due to fusion or splitting of domains during evolution and are not considered to be mechanistic differences (Postma, P.W. *et al., Microbiol. Rev. 57*:543 (1993)). In *M. genitalium* EIIA, B, and C are located in a single protein similar to the protein found in *B. subtilis*. In *Mycoplasma capricolum ptsH*, the gene which encodes for HPr, is located on a monocistronic transcriptional unit while genes encoding EI (*ptsI*) and EIIA (crr) are located on a dicistronic operon (Zhu, P.P. *et al., Protein Sci. 3*:2115 (1994); Zhu, P.P. *et al., J. Biol. Chem. 268*:26531 (1993)). In most bacterial species studied to date, *ptsI, ptsH*, and *crr* are part of a polycistronic operon (*pts* operon). In *M. genitalium ptsH, ptsI* and the gene encoding EIIABC reside at different locations of the genome and thus each of these genes may constitute monocistronic transcriptional units. We have also identified EIIBC component for uptake of fructose; however, other components of the fructose PTS were not found. Thus, *M. genitalium* may be limited to the use of glucose as a energy source. In contrast, *H. influenzae* has the ability to use at least six different sugars as a source of carbon and energy.

Regulatory Systems. It appears that regulatory systems found in other bacteria are absent in *M. genitalium*. For instance, although two component systems have been described for a number of gram-positive organisms, no sensor or response regulator genes are found in the *M. genitalium* genome. Furthermore, the lack of a heat shock σ factor raises the question of how the heat shock response is regulated. Another stress faced by all metabolically active organisms is the generation of reactive oxygen intermediates such as superoxide anions and hydrogen peroxide. Although *H. influenzae* has a *oxyR* homologue, as well as catalase and superoxide dismutase, *M. genitalium* appears to lack these genes as well as an NADH peroxidase. The importance of these reactive intermediate molecules in host cell damage suggests that some as yet unidentified protective mechanism may exist within the cell.

Antigenic variation. Numerous examples exist of microbial pathogens expressing outer membrane proteins that vary due to DNA rearrangements as a mechanism for providing antigenic and functional variations that influence virulence potential (Bergstrom, S. *et al., Proc. Natl. Acad. Sci. USA 83*:3890 (1986); Meier, J.T. *et al., Cell 47*:61 (1986); Majiwa, P.A.O. *et al., Nature* 297:514 (1982)). Because humans are the natural host for both *M. genitalium* and *H. influenzae*, it was of interest to compare mechanisms for generating antigenic variation in these organisms. In *H. influenzae*, a number of virulence-related genes encoding membrane proteins contain tandem tetramer repeats that undergo frequent addition and deletion of one or more repeat units during replication, such that the reading frame of the gene is changed and its expression altered (Weiser, J.N. *et al., Cell 59*:657 (1989)).

M. genitalium appears to use a different system for evading host immune responses. The 140 kDa adhesion protein of *M. genitalium* is densely clustered at a differentiated tip of this organism and elicits a strong immune response in humans and experimentally infected animals (Collier, A.M. *et al., Zbl. Bkt. Suppl. 20*:73 (1992)). The adhesion protein (MgPa) operon in *M. genitalium* contains a 29 kDa ORF, the MgPa protein (160 kDa) and a 114 kDa ORF with intervening regions of 6 and 1 nt, respectively (Inamine, J.M. *et al., Gene 82*:259 (1989)). Based on hybridization experiments (Dallo, S.F. and Baseman, J.B., *Microb. Pathog. 8*:371 (1990)), multiple copies of regions of the *M. genitalium* MgPa gene and the 114 kDa ORF are known to exist throughout the genome.

The availability of the complete genomic sequence from *M. genitalium* has allowed a comprehensive mapping of the MgPa repeats (Figures 4 and 6). In addition to the complete operon, nine repetitive elements which are composites of particular regions of the MgPa operon were found. The percent of sequence identity between the repeat elements and the MgPa gene ranges from 78%-90%. In some of the repeats, the MgPa-related sequences are separated in the genome by a variable length, A-T rich spacer sequence, as has previously been described (Peterson, S.N., PhD dissertation, Univ. No. Carolina 1992, Univ. Mi. Dissertation Services #6246). The sequences contained in the MgPa operon ad the nine repeats scattered throughout the chromosome represent 4.5% of the total genomic sequence. At first glance this might appear to contradict the expectation for a minimal genome. However, recent evidence for recombination between the repetitive elements and the MgPa operon has been reported (Peterson, S.N. *et al.*, *Proc. Natl. Acad Sci. USA*, in press (1995)). Such recombination may allow *M. genitalium* to evade the host immune response through mechanisms that induce antigenic variation within the population. Since *M. genitalium* survives in nature by obtaining essential nutrients from its mammalian host, a efficient mechanism to evade the immune response may be a necessary part of this minimal genome.

The *M. genitalium* genome contains 93 putatively identified genes that are apparently not present in *H. influenzae*. Almost 60% of these genes have database matches to known or hypothetical proteins from gram-positive bacteria or other *Mycoplasma* species, suggesting that these genes may encode proteins with a restricted phylogenetic distribution. One hundred seventeen potential coding regions in *M. genitalium* have no database match to any sequences in public archives including the entire *H. influenzae* genome; therefore, these likely represent novel. genes in *M. genitalium*, and related organisms.

The predicted coding sequences of the hypothetical ORFs, the ORFs with motif matches and the ORFs that have no similarities to known peptide sequences were analyzed. The two programs used were the Kyte-Doolittle algorithm (Kyte, J. and Doolittle, R.F., *J. Mol. Biol. 157*:105 (1982)) with a range of 11 residues, and PSORT which is available on the WWW site http://psort.nibb.ac.jp. PSORT predicts the presence of signal sequences by the methods of McGeoch (McGeoch, D.J., *Virus Res. 3*:271 (1985)) and von Heijne (von Heijne, G., *Nucl. Acids Res. 14*:4683 (1986)), and detects potential transmembrane domains by the method of Klein *et al.* (Klein, P. *et al.*, *Biochim. Biophys. Acta 815*:468 (1985)). Of a total of 201 ORFs examined, 90 potential membrane proteins were found. Eleven of them are predicted to have type I signal peptides, ad five type II signal peptides. Using this approach, at least fifty potential membrane proteins were identified from the list of ORFs with known functions. This brings the total number of membrane proteins in *M. genitalium* to approximately 140.

To manage these putative membrane proteins, *M. genitalium* has at its disposal a minimal secretary machinery composed of seven functions: three chaperoning GroEL, DnaK ad the trigger factor Tig (Pugsley, A.P., *Microbiol. Rev. 57*:50 (1993); Guthrie, B. and Wickner, W., *J. Bacteriol. 172*:5555 (1990), an ATPase pilot protein SecA, one integral membrane protein translocase (SecY), a signal recognition particle protein (Ffh) and a lipoprotein-specific signal peptidase LspA (Pugsley, A.P., *Microbiol. Rev. 57*:50 (1993)). Perhaps the lack of other known translocases like SecE, SecD, and SecF which are present in *E. coli* and *H. influenzae*, is related to the fact that *M. genitalium* has a one-layer cell envelope. Also, the absence of a SecB homologue, the secretory chaperonin of *E. coli*, in *M. genitalium* (it is also absent in *B. subtilis* (Collier, D.N. *J. Bacteriol. 176*:4937 (1994))) might reflect a difference between gram negative ad wall-less Mollicutes in handling nascent proteins destined for the general secretory pathway. Considering the presence of several putative membrane proteins that contain type I signal peptides, the absence of a signal peptidase I (*lepB*) is most surprising. A direct electronic search for the *M. genitalium lepB* gene using the *E. coli lepB* and the *B. subtilis sipS* (van Dijil, J.M. *et al.*, *EMBO J. 11*:2819 (1992)) as queries did not reveal any significant similarities.

There are a number of possible explanations as to why genes encoding some of the proteins thought to be essential for a self-replicating organism appear to be absent in *M. genitalium*. One possibility is that a limited number of proteins may have adapted to take on other functions. A second possibility is that certain proteins thought to be essential for life based on studies in *E. coli* are not required in a simpler prokaryote like *M. genitalium*. Finally, it may be that sequences from *M. genitalium* have such a low similarity to known sequences from other species that matches are not detectable above a reasonable confidence threshold.

Determination of the complete genome sequence of *M. genitalium* provides a new starting point in understanding the biology of this ad related organisms. Comparison of the genes expressed in *M. genitalium*, a simple prokaryote, with those in *H. influenzae*, a more complex organism, has revealed a myriad of differences between these species. Fifty-six percent of the genes in *M. genitalium* have apparent isologs in *H. influenzae*, suggesting that this subset of the *M. genitalium* genome may encode the genes that are truly essential for a self-replicating organism. Notable among the genes that are conserved between *M. genitalium* and *H. influenzae* are those involved in DNA replication ad repair, transcription and translation, cell division, and basic energy metabolism via glycolysis. Isologs of these genes are found in eukaryotes as well.

Example 2

Production of an Antibody to a Mycoplasma genitalium Protein

Substantially pure protein or polypeptide is isolated from the transfected or transformed cells using any one of the methods known in the art. The protein can also be produced in a recombinant prokaryotic expression system, such as *E. coli*, or can by chemically synthesized. Concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., *Nature 256*:495 (1975) or modifications of the methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted ad aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., *Meth. Enzymol. 70*:419 (1980), and modified methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. *et al.* Basic Methods in Molecular Biology Elsevier, New York. Section 21-2 (1989).

Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. *et al.*, *J. Clin. Endocrinol. Metab. 33*:988-991 (1971).

Booster injections can be given at regular intervals, ad antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall (*See* Ouchterlony, O. *et al.*, Chap. 19 in: *Handbook of Experimental Immunology*, Wier, D., ed, Blackwell (1973)). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 $_{\mu}$M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: *Manual of Clinical Immunology*, second edition, Rose and Friedman, (eds.), Amer. Soc. For Microbio., Washington, D.C. (1980).

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample.

Example 3

Preparation of PCR Primers and Amplification of DNA

Various fragments of the *Mycoplasma genitalium* genome, such as those disclosed in Tables 1a, 1b, 1c and 2 can be used, in accordance with the present invention, to prepare PCR primers for a variety of uses. The PCR primers are preferably at least 15 bases, and more preferably at least 18 bases in length. When selecting a primer sequence, it is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. The PCR primers and amplified DNA of this Example find use in the examples that follow.

Example 4

Gene expression from DNA Sequences Corresponding to ORFs

A fragment of the *Mycoplasma genitalium* genome provided in Tables 1a, 1b, 1c and 2 is introduced into a expression vector using conventional technology (techniques to transfer cloned sequences into expression vectors that direct protein translation in mammalian, yeast, insect or bacterial expression systems are well known in the art). Commercially available vectors ad expression systems are available from a variety of suppliers including Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism, as explained by Hatfield *et al.*, U.S. Pat. No. 5,082,767, which is hereby incorporated by reference.

The following is provided as one exemplary method to generate polypeptide(s) from cloned ORFs of the *Mycoplasma* genome fragment. Since the ORF lacks a poly A sequence because of the bacterial origin of the ORF, this sequence can be added to the construct by, for example, splicing out the poly A sequence from pSG5 (Stratagene) using *Bgl*I and *Sal*I restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene) for use in eukaryotic expression systems. pXT1 contains the LTRs and a portion of the gag gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex thymidine kinase promoter ad the selectable neomycin gene. The *Mycoplasma* DNA is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the *Mycoplasma* DNA and containing restriction endonuclease sequences for *Pst*I incorporated into the 5' primer and *Bgl*II at the 5' end of the corresponding *Mycoplasma* DNA 3' primer, taking care to ensure that the *Mycoplasma* DNA is positioned such that its followed with the poly A sequence. The purified fragment obtained from the resulting PCR reaction is digested with *Pst*I, blunt ended with a exonuclease, digested with *Bgl*II, purified and ligated to pXT1, now containing a poly A sequence and digested *Bgl*II.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 ug/ml G418 (Sigma, St. Louis, Missouri). The protein is preferably released into the supernatant. However if the protein has membrane binding domains, the protein may additionally be retained within the cell or expression may be restricted to the cell surface.

Since it may be necessary to purify and locate the transfected product, synthetic 15-mer peptides synthesized from the predicted *Mycoplasma* DNA sequence are injected into mice to generate antibody to the polypeptide encoded by the *Mycoplasma* DNA.

If antibody production is not possible, the *Mycoplasma* DNA sequence is additionally incorporated into eukaryotic expression vectors and expressed as a chimeric with, for example, β-globin. Antibody to β-globin is used to purify the chimeric. Corresponding protease cleavage sites engineered between the β-globin gene and the *Mycoplasma* DNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating β-globin chimerics is pSG5 (Stratagene). This vector encodes rabbit β-globin. Intron II of the rabbit β-globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques as described are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis et al. and may of the methods are available from the technical assistance representatives from Stratagene, Life Technologies, Inc., or Promega. Polypeptide may additionally be produced from either construct using in vitro translation systems such as In vitro Express™ Translation Kit (Stratagene).

Table 1(a)

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG006	8552	9181	SP-P00572	thymidylate kinase (CDC8) {Saccharomyces cerevisiae}	27.5862	51.7241	630
MG009	11252	12037	GB:D26185_102	hypothetical protein (GB:D26185_102) {Bacillus subtilis}	35.4331	55.1181	786
MG010	12069	12722	SP:P33655	DNA primase (dnaE) {Clostridium acetobutylicum}	25.731	53.2164	654
MG012	14247	13573	SP:P17116	ribosomal protein S6 modification protein (rimK) {Escherichia coli}	31.4961	54.3307	675
MG013	15217	14399	GB:D10588_1	5,10-methylene-tetrahydrofolate dehydrogenase (folD) {Escherichia coli}	33.0472	53.2189	819
MG015	17474	19240	SP:P27299	transport ATP-binding protein (msbA) {Escherichia coli}	32.2382	57.4949	1767
MG023	26478	27341	GB:M22039_4	fructose-bisphosphate aldolase (tsr) {Bacillus subtilis}	45.9649	65.9649	864
MG024	27345	28445	GP:U02423_1	GTP-binding protein (gtp1) {Escherichia coli}	46.8401	67.658	1101
MG032	36978	38975	GB:M63489_1	ATP-dependent nuclease (addA) {Bacillus subtilis}	26.8293	54.2683	1998
MG033	39242	39901	GB:M99611_2	glycerol uptake facilitator (glpF) {Bacillus subtilis}	35.8974	55.3846	660
MG034	40514	39876	GB:M97678_5	thymidine kinase (tdk) {Bacillus subtilis}	48.1283	69.5187	639
MG035	40543	41784	GB:U00011_2	histidyl-tRNA synthetase (hisS) {Mycobacterium leprae}	30.7107	50.7614	1242

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG038	46277	44754	GB:L19201_68	glycerol kinase (glpK) {Escherichia coli}	46.8254	70.2381	1524
MG039	47422	46271	PIR:S48379	glycerol-3-phospate dehydrogenase (GUT2) {Saccharomyces cerevisiae}	43.2099	60.4938	1152
MG041	49377	49640	GB:L22432_2	phosphohistidinoprotein-hexose phosphotransferase (ptsH) {Mycoplasma capricolum}	48.8636	70.4545	264
MG042	50060	51517	GB:M64519_1	spermidine/putrescine transport ATP-binding protein (potA) {Escherichia coli}	41.9231	65.3846	1458
MG043	51525	52379	GB:M64519_2	spermidine/putrescine transport system permease protein (potB) {Escherichia coli}	26.5116	57.2093	855
MG044	52366	53217	GB:M64519_3	spermidine/putrescine transport system permease protein (potC) {Escherichia coli}	29.4574	58.1395	852
MG046	54658	55602	GB:M62364_1	sialoglycoprotease (gcp) {Pasteurella haemolytica}	36.6013	59.4771	945
MG048	58310	56973	SP:P37105	signal recognition particle protein (ffh) {Bacillus subtilis}	43.0206	66.1327	1338
MG049	58117	59076	GB:U14003_295	purine-nucleoside phosphorylase (deoD) {Escherichia coli}	44.7826	63.0435	960
MG050	59083	59751	GB:X13544_1	deoxyribose-phosphate aldolase (deoC) {Mycoplasma pneumoniae}	83.0357	91.5179	669

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG056	65731	64901	GB:D26185_99	hypothetical protein (GB:D26185_99) {Bacillus subtilis}	30.2583	54.6125	831
MG057	66249	65716	GB:D26185_104	hypothetical protein (GB:D26185_104) {Bacillus subtilis}	28.9017	28.9017	534
MG067	81047	82594	GB:D00730_1	glutamic acid specific protease (SPase) {Staphylococcus aureus}	28.8462	48.0769	1548
MG070	91065	91916	SP:P34831	ribosomal protein S2 (rpS2) {Spirulina platensis}	34.8	55.2	852
MG077	103104	104324	SP:P24138	oligopeptide transport system permease protein (oppB) {Bacillus subtilis}	28.0528	58.4158	1221
MG078	104320	105447	SP:P26904	oligopeptide transport system permease protein (dciAC) {Bacillus subtilis}	33.4572	55.0186	1128
MG079	105452	106657	SP:P18765	oligopeptide transport ATP-binding protein (amiE) {Streptococcus pneumoniae}	47.9412	67.9412	1206
MG081	109262	109672	SP:P29395	ribosomal protein L11 (RPL11) {Thermotoga maritima}	51.7986	71.9424	411
MG085	111790	112722	PIR:S24760	hydroxymethylglutaryl-CoA reductase (NADPH) {Nicotiana sylvestris}	23.3216	49.1166	933
MG086	112718	113863	GB:L13259_2	prolipoprotein diacylglyceryl transferase (lgt) {Salmonella typhimurium}	29.1262	53.8835	1146

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG091	117553	118032	GB:U04997_2	single-stranded DNA binding protein (ssb) {Haemophilus influenzae}	21.7949	41.6667	480
MG092	118025	118339	GB:U14003_114	ribosomal protein S18 (rpS18) {Escherichia coli}	45.4545	68.1818	315
MG093	118345	118794	GB:M57623_1	ribosomal protein L9 (rpL9) {Bacillus stearothermophilus}	32.8859	56.3758	450
MG099	125852	127282	GB:M61151_1	hydrolase (aux2) {Agrobacterium rhizogenes}	32.1212	51.8182	1431
MG106	134826	134149	SP:P27251	formylmethionine deformylase (def) {Escherichia coli}	36.9369	68.4685	678
MG107	134558	135334	GB:L10328_14	5'guanylate kinase (gmk) {Escherichia coli}	42.623	65.0273	777
MG114	141345	142052	GB:M12299_2	phosphatidylglycerophosphate synthase (pgsA) {Escherichia coli}	29.2994	57.3248	708
MG118	143935	144954	SP:P09147	UDP-glucose 4-epimerase (galE) {Escherichia coli}	34.0557	53.87	1020
MG121	148238	149155	SP:P32720	hypothetical protein (SP:P32720) {Escherichia coli}	30.8824	50.7353	918

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG125	153081	153935	GB:L10328_61	hypothetical protein (GB:L10328_61) {Escherichia coli}	31.9149	48.227	855
MG126	154962	153922	GB:M24068_1	tryptophanyl-tRNA synthetase (trpS) {Bacillus subtilis}	41.1585	61.5854	1041
MG127	154998	155432	SP:P19434	hypothetical protein (SP:P19434) {Streptomyces viridochromogenes}	25.9615	49.0385	435
MG128	155443	156219	GB:U00021_19	hypothetical protein (GB:U00021_19) {Mycobacterium leprae}	27.7027	49.3243	777
MG129	156222	156572	GB:U12340_1	PTS glucose-specific permease {Bacillus stearothermophilus}	25.4545	51.8182	351
MG130	156565	158016	GB:M91593_1	hypothetical protein (GB:M91593_1) {Mycoplasma mycoides}	30.6773	55.7769	1452
MG131	158022	158243	GB:M31161_3	hypothetical protein (GB:M31161_3) {Spiroplasma citri}	21.5909	56.8182	222
MG132	159005	158583	SP:P32083	hypothetical protein (SP:P32083) {Mycoplasma hyorhinis}	30.0971	56.3107	423
MG136	160962	162431	GB:D26185_144	lysyl-tRNA synthetase (lysS) {Bacillus subtilis}	45.6212	68.4318	1470

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG137	162376	163587	GP:L41518_4	dTDP-4-dehydrorhamnose reductase (rfbD) {Klebsiella pneumoniae}	32.1622	55.9459	1212
MG139	165470	167176	GB:L18927_2	hypothetical protein (GB:L18927_2) {Buchnera aphidicola}	28.5714	62.8571	1707
MG143	182853	183188	SP:P09170	hypothetical protein (SP:P09170) {Escherichia coli}	25	53.7037	336
MG145	184055	184861	GB:M35367_1	protein X {Pseudomonas fluorescens}	29.0698	48.4496	807
MG148	187304	188530	GB:L18965_6	hypothetical protein (GB:L18965_6) {Thermophilic bacterial sp.}	25.2874	52.8736	1227
MG150	190048	190365	SP:P38518	ribosomal protein S10 (rpS10) {Thermotoga maritima}	48.913	71.7391	318
MG152	191145	191777	SP:P28601	ribosomal protein L4 (rpL4) {Bacillus stearothermophilus}	39.2345	63.1579	633
MG153	191784	192101	SP:P04454	ribosomal protein L23 (rpL23) {Bacillus stearothermophilus}	38.7097	62.3656	318
MG154	192104	192958	SP:P04257	ribosomal protein L2 (rpL2) {Bacillus stearothermophilus}	58.7814	72.4014	855

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG155	192961	193221	GB:X02613_6	ribosomal protein S19 (rpS19) {Escherichia coli}	58.6207	77.0115	261
MG156	193227	193658	GB:M74770_4	ribosomal protein L22 (rpL22) {Mycoplasma-like organism}	49.0385	67.3077	432
MG157	193664	194467	SP:P02353	ribosomal protein S3 (rpS3) {Mycoplasma capricolum}	46.729	67.2897	804
MG158	194476	194889	SP:P02415	ribosomal protein L16 (rpL16) {Mycoplasma capricolum}	63.5037	78.1022	414
MG159	194892	195491	SP:P38514	ribosomal protein L29 (rpL29) {Thermotoga maritima}	41.6667	65	600
MG160	195494	195748	SP:P10131	ribosomal protein S17 (rpS17) {Mycoplasma capricolum}	51.1905	67.8571	255
MG161	195755	196120	SP:P04450	ribosomal protein L14 (rpL14) {Bacillus stearothermophilus}	63.1148	86.0656	366
MG162	196123	196446	SP:P04455	ribosomal protein L24 (rpL24) {Bacillus stearothermophilus}	44.5783	66.2651	324
MG163	196455	196994	SP:P08895	ribosomal protein L5 (rpL5) {Bacillus stearothermophilus}	57.5419	77.095	540

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG164	197000	197182	GB:X06414_15	ribosomal protein S14 (rpS14) {Mycoplasma capricolum}	70.4918	83.6066	183
MG165	197179	197601	SP:P04446	ribosomal protein S8 (rpS8) {Mycoplasma capricolum}	46.875	71.0938	423
MG166	197611	198162	SP:P04448	ribosomal protein L6 (rpL6) {Mycoplasma capricolum}	46.9945	66.6667	552
MG167	198167	198511	GB:M57624_1	ribosomal protein L18 (rpL18) {Bacillus stearothermophilus}	42.9825	57.8947	345
MG169	199160	199609	SP:P10138	ribosomal protein L15 (rpL15) {Mycoplasma capricolum}	41.8919	66.2162	450
MG170	199612	201036	SP:P10250	preprotein translocase secY subunit (secY) {Mycoplasma capricolum}	38.7892	68.1614	1425
MG171	201033	201674	GB:M88104_2	adenylate kinase (adk) {Bacillus stearothermophilus}	32.2115	57.6923	642
MG172	201680	202423	GB:D00619_5	methionine amino peptidase (map) {Bacillus subtilis}	36.2903	58.4677	744
MG173	202426	202635	GB:M26414_1	initiation factor 1 (infA) {Bacillus subtilis}	48.5294	67.6471	210

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG174	202649	202759	SP:P38015	ribosomal protein L36 (rpL36) {Chlamydia trachomatis}	78.3784	83.7838	111
MG177	203516	204499	GB:M26414_5	RNA polymerase alpha core subunit (rpoA) {Bacillus subtilis}	39.3939	65.9933	984
MG178	204515	204515	GB:M26414_6	ribosomal protein L17 (rpL17) {Bacillus subtilis}	34.7826	59.1304	369
MG179	204873	205694	SP:P11599	haemolysin secretion ATP-binding protein (hlyB) {Proteus vulgaris}	34.5992	62.0253	822
MG187	216762	218516	GB:M77351_7	ATP-binding protein (msmK) {Streptococcus mutans}	40.5325	65.6805	1755
MG188	218522	219508	GB:M77351_4	membrane protein (msmF) {Streptococcus mutans}	22.4719	51.6854	987
MG189	219435	220436	GB:M77351_5	membrane protein (msmG) {Streptococcus mutans}	27.1429	52.8571	1002
MG196	235635	236057	GB:X16188_1	translation initiation factor IF3 (infC) {Bacillus stearothermophilus}	31.3433	62.6866	423
MG197	236063	236239	PIR:S05347	ribosomal protein L35 (rpL35) {Bacillus stearothermophilus}	60	72.7273	177

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG198	236245	236616	SP:Q05427	ribosomal protein L20 (rpL20) {Mycoplasma fermentans}	57.5221	73.4513	372
MG201	239163	239813	GB:M84964_2	heat shock protein (grpE) {Bacillus subtilis}	31.677	49.6894	651
MG205	245596	244568	GB:M84964_1	hypothetical protein (GB:M84964_1) {Bacillus subtilis}	30.9942	58.1871	1029
MG213	252579	253991	GB:L09228_16	hypothetical protein (GB:L09228_16) {Bacillus subtilis}	27.1186	54.661	1413
MG214	253978	254598	GB:L09228_17	hypothetical protein (GB:L09228_17) {Bacillus subtilis}	34.8571	59.4286	621
MG215	254620	255588	SP:P20275	6-phosphofructokinase (pfk) {Spiroplasma citri}	39.441	63.0435	969
MG217	258040	259155	SP:P29126	bifunctional endo-1,4-beta-xylanase xyla precursor (xynA) {Ruminococcus flavefaciens}	37.5839	48.9933	1116
MG219	265596	266039	GB:M87491_1	IgA1 protease {Haemophilus influenzae}	32.2314	51.2397	444
MG220	266382	266077	GB:Z26883_1	pre-procytotoxin (vacA) {Helicobacter pylori}	36.1446	51.8072	306

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG222	267080	268006	GB:D10483_63	hypothetical protein (GB:D10483_63) {Escherichia coli}	35.1974	56.5789	927
MG224	269249	270355	GB:U06462_1	cell division protein (ftsZ) {Staphylococcus aureus}	30.8824	50.7353	1107
MG234	279491	279802	GB:K02665_2	ribosomal protein L27 (rpL27) {Bacillus subtilis}	64.3678	80.4598	312
MG235	279798	280670	SP:P12638	endonuclease IV (nfo) {Escherichia coli}	29.368	51.3011	873
MG245	293446	293940	GB:M12965_1	hypothetical protein (GB:M12965_1) {Escherichia coli}	33.8462	56.9231	495
MG247	295484	294768	SP:P31056	hypothetical protein (SP:P31056) {Escherichia coli}	32.973	56.2162	717
MG248	296127	295474	GP:U17284_2	major sigma factor (rpoD) {Listeria monocytogenes}	28.4848	51.5152	654
MG251	300802	299465	GB:L08106_1	glycyl-tRNA synthetase {Bombyx mori}	35.8974	56.1772	1338
MG252	301550	300825	GP:Z33076_2	rRNA methylase {Mycoplasma capricolum}	38.8626	59.7156	726

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG253	302839	301556	GB:D26185_156	cysteinyl-tRNA synthetase (cysS) {Bacillus subtilis}	34.3458	56.3084	1284
MG257	307635	307925	GB:L19201_78	ribosomal protein L31 (rpL31) {Escherichia coli}	37.3134	61.194	291
MG258	307928	309004	GB:M11519_1	peptide chain release factor 1 (RF-1) {Escherichia coli}	43.1677	66.4596	1077
MG259	309008	310375	GB:D28567_2	protoporphyrinogen oxidase (hemK) {Escherichia coli}	30.5732	54.1401	1368
MG260	310509	312803	GB:Z32651_1	hypothetical protein (GB:Z32651_1) {Mycoplasma pneumoniae}	57.1429	71.4286	2295
MG262	318330	319202	GB:L11920_1	DNA polymerase I (polI) {Mycobacterium tuberculosis}	29.9419	47.9651	873
MG264	321044	321637	GB:M64324_1	6-phosphogluconate dehydrogenase (gnd) {Escherichia coli}	29.8507	47.7612	594
MG265	322412	321579	GB:L10328_61	hypothetical protein (GB:L10328_61) {Escherichia coli}	27.193	48.6842	834
MG268	325877	325194	GB:U01881_2	deoxyguanosine/deoxyadenosine kinase(I) subunit 2 {Lactobacillus acidophilus}	29.5181	49.3976	684

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG270	328442	327435	GB:U14003_297	hypothetical protein (GB:U14003_297) {Escherichia coli}	38.2838	57.7558	1008
MG272	330984	329833	GB:M81753_3	dihydrolipoamide acetyltransferase (pdhC) {Acholeplasma laidlawii}	45.1524	62.0499	1152
MG273	332214	331237	GB:M81753_2	pyruvate dehydrogenase E1-beta subunit (pdhB) {Acholeplasma laidlawii}	55.0314	76.7296	978
MG274	333308	332235	GB:M81753_1	pyruvate dehydrogenase E1-alpha subunit (pdhA) {Acholeplasma laidlawii}	42.9825	61.1111	1074
MG277	338323	335414	GB:L16960_2	spore germination apparatus protein (gerBB) {Bacillus subtilis}	31.2	55.2	2910
MG280	341920	341177	GB:Z35086_1	sensory rhodopsin II transducer (htrII) {Natronobacterium pharaonis}	15.7143	46.6667	744
MG288	353034	351793	GB:L04466_1	protein L {Peptostreptococcus magnus}	31.1475	50.8197	1242
MG290	355119	355853	SP:P15361	ATP-binding protein P29 {Mycoplasma hyorhinis}	32.3009	58.8496	735
MG292	360592	357893	GB:J01581_1	alanyl-tRNA synthetase (alaS) {Escherichia coli}	33.8403	55.64	2700

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG295	364022	362922	SP:P25745	hypothetical protein (SP:P25745) {Escherichia coli}	34.7107	57.0248	1101
MG299	369694	368735	SP:P39646	phosphotransacetylase (pta) {Clostridium acetobutylicum}	44.6541	63.522	960
MG303	373998	372928	GB:M61017_1	membrane transport protein (glnQ) {Bacillus stearothermophilus}	31.982	54.955	1071
MG304	374741	373983	GB:U13043_1	membrane associated ATPase (cbiO) {Propionibacterium freudenreichii}	30.0448	53.8117	759
MG310	386462	387265	GB:D11037_1	proline iminopeptidase (pip) {Bacillus coagulans}	29.2079	51.4851	804
MG311	387892	387278	GB:M59358_1	ribosomal protein S4 (rpS4) {Bacillus subtilis}	43	65.5	615
MG313	392023	391397	GP:L38997_5	cytadherence-accessory protein (hmw1) {Mycoplasma pneumoniae}	53.8462	79.8077	627
MG315	394550	393660	GP:L38997_3	cytadherence accessory protein (hmw1) {Mycoplasma pneumoniae}	44.3878	69.898	891
MG316	395583	394477	GB:L15202_4	competence locus E (comE3) {Bacillus subtilis}	30.4933	52.4664	1107

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG322	405398	403725	GB:D17462_11	Na+ ATPase subunit J (ntpJ) {Enterococcus hirae}	31.0811	56.3063	1674
MG323	405455	406135	GB:D37799_6	hypothetical protein (GB:D37799_6) {Bacillus subtilis}	27.5701	54.2056	681
MG325	408953	408795	SP:P23375	ribosomal protein L33 (rpL33) {Bacillus stearothermophilus}	58.1395	69.7674	159
MG326	409857	408973	GB:Z18629_1	hypothetical protein (GB:Z18629_1) {Bacillus subtilis}	27.0758	52.7076	885
MG329	414318	412975	GB:U00021_5	hypothetical protein (GB:U00021_5) {Mycobacterium leprae}	32.1839	54.2529	1344
MG332	416329	415613	GB:D10165_3	hypothetical protein (GB:D10165_3) {Escherichia coli}	26.9231	49.1453	717
MG346	443922	444419	GB:M65289_3	hypothetical protein (GB:M65289_3) {Bacillus stearothermophilus}	37.9747	60.1266	498
MG347	444413	445042	SP:P32049	hypothetical protein (SP:P32049) {Escherichia coli}	28.4615	46.9231	630
MG351	449665	450216	SP:P37981	inorganic pyrophosphatase (ppa) {Thermoplasma acidophilum}	38.8535	61.7834	552

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG355	453757	451616	GB:M29364_2	ATP-dependent protease binding subunit (clpB) {Escherichia coli}	47.7337	70.6799	2142
MG356	454753	453914	GB:M27280_1	lic-1 operon protein (licA) {Haemophilus influenzae}	27.7778	56.25	840
MG359	457347	458267	GB:M21298_2	Holliday junction DNA helicase (ruvB) {Escherichia coli}	34.6939	64.966	921
MG360	459495	458263	SP:P14303	UV protection protein (mucB) {Salmonella typhimurium}	22.0859	48.1595	1233
MG363	460497	460667	GB:M29698_2	ribosomal protein L32 (rpL32) {Escherichia coli}	48.1481	62.963	171
MG364	461015	461686	GB:M95954_1	mobilization protein (mob13) {Leuconostoc oenos}	30.8725	53.6913	672
MG367	465434	464649	GB:X02673_1	ribonuclease III (rnc) {Escherichia coli}	30.1724	65.5172	786
MG380	478999	479574	GB:L10328_105	glucose inhibited division protein (gidB) {Escherichia coli}	24.8276	51.7241	576
MG382	480691	481329	SP:P31218	uridine kinase (udk) {Escherichia coli}	34.4828	62.5616	639

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG383	482075	481332	GB:M15811_1	sporulation protein (outB) {Bacillus subtilis}	36.3636	54.9784	744
MG384	483369	482071	GB:M24537_2	GTP-binding protein (obg) {Bacillus subtilis}	39.627	62.0047	1299
MG387	490711	489842	SP:P37214	GTP-binding protein era homolog (spg) {Streptococcus mutans}	27.3859	51.0373	870
MG396	500719	500264	GB:M80797_2	galactosidase acetyltransferase (lacA) {Streptococcus mutans}	40.5797	57.971	456
MG398	502823	502425	SP:P33255	ATP synthase epsilon chain (atpC) {Mycoplasma gallisepticum}	36.9231	55.3846	399
MG402	507201	506674	SP:P33254	ATP synthase delta chain (atpH) {Mycoplasma gallisepticum}	33.9181	58.4795	528
MG403	507820	507197	SP:P33256	ATP synthase B chain (atpF) {Mycoplasma gallisepticum}	36.5979	66.4948	624
MG404	508131	507826	SP:P33258	ATP synthase C chain (atpE) {Mycoplasma gallisepticum}	50	74.359	306
MG407	510836	509463	GB:L29475_4	enolase (eno) {Bacillus subtilis}	54.0793	74.1259	1374

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG408	510903	511373	SP:P14930	pilin repressor (pilB) {Neisseria gonorrhoeae}	49.2188	68.75	471
MG409	512050	511376	GB:L10328_88	peripheral membrane protein U (phoU) {Escherichia coli}	27.027	48.6486	675
MG420	524144	523365	GB:D26185_83	DNA polymerase III subunit (dnaH) {Bacillus subtilis}	49.115	68.5841	780
MG424	531479	531222	SP:P05766	ribosomal protein S15 (BS18) {Bacillus stearothermophilus}	48.1481	71.6049	258
MG426	533040	533231	GB:L12244_2	ribosomal protein L28 (rpL28) {Bacillus subtilis}	36.0656	59.0164	192
MG429	536036	534321	GB:M69050_2	PEP-dependent HPr protein kinase phosphoryltransferase (ptsI) {Staphylococcus carnosus}	46.4789	66.5493	1716
MG430	537563	536043	GB:L29475_3	phosphoglycerate mutase (pgm) {Bacillus subtilis}	45.1866	62.4754	1521
MG432	539546	538353	SP:P27712	hypothetical protein (SP:P27712) {Spiroplasma citri}	28.436	48.8152	1194
MG433	539632	540525	GB:M31161_2	elongation factor Ts (tsf) {Spiroplasma citri}	39.0572	62.6263	894

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG434	540848	541237	GB:D26562_56	mukB suppressor protein (smbA) {Escherichia coli}	40.8696	61.7391	390
MG435	541240	541788	GB:D26562_57	ribosome releasing factor (frr) {Escherichia coli}	34.9112	57.3965	549
MG438	543004	544152	GB:J01631_1	restriction-modification enzyme EcoD specificity subunit (hsdS) {Escherichia coli}	24.5734	45.7338	1149
MG442	547690	546881	GB:U00021_5	hypothetical protein (GB:U00021_5) {Mycobacterium leprae}	26.8966	42.069	810
MG443	548849	547665	GB:D16311_1	hypothetical protein (GB:D16311_1) {Bacillus subtilis}	26.1818	52	1185
MG444	549224	548868	SP:P30529	ribosomal protein L19 (rpL19) {Bacillus stearothermophilus}	49.1071	69.6429	357
MG445	549903	549211	SP:P36245	tRNA (guanine-N1)-methyltransferase (trmD) {Salmonella typhimurium}	40.8072	64.1256	693
MG446	550172	549906	SP:P21474	ribosomal protein S16 (BS17) {Bacillus subtilis}	48.7805	64.6341	267
MG448	552897	552448	GB:Z33052_1	pilin repressor (pilB) {Mycoplasma capricolum}	53.4884	72.093	450

UID	end5	end3	db_match	db_match name	per_id	per_sim	gene_len
MG454	557770	557306	SP:P23929	osmotically inducible protein (osmC) {Escherichia coli}	28.4091	51.1364	465
MG457	562602	560497	GB:D26185_132	cell division protein (ftsH) {Bacillus subtilis}	49.7445	68.1431	2106
MG461	566203	564929	GB:X73124_94	hypothetical protein (GB:X73124_94) {Bacillus subtilis}	40	64.2857	1275
MG464	569554	568400	GB:D14982_3	hypothetical protein (GB:D14982_3) {Mycoplasma capricolum}	32.3699	53.7572	1155
MG465	569912	569529	GB:D14982_2	RNaseP C5 subunit (rnpA) {Mycoplasma capricolum}	40	58.75	384
MG466	570027	569884	GB:L10328_67	ribosomal protein L34 (rpL34) {Escherichia coli}	67.3913	80.4348	144
MG470	580030	579224	GB:D26185_55	SpoOJ regulator {Bacillus subtilis}	27.8884	53.3865	807

Table 1(b)

UID	end5	end3	db_match	db_match name	per_sim	per_id	match_info
MG002	1829	2758	SP:P35514	heat shock protein (dnaJ) {Lactococcus lactis}	40	61.6667	MG002(1 - 930 of 930) GB:U09251(298 - 1227 of 6140)
MG003	2846	4795	GB:U09251_3	DNA gyrase subunit B (gyrB) {Mycoplasma genitalium}	99.3846	99.3846	MG003(1 - 1950 of 1950) GB:U09251(1315 - 3264 of 6140)
MG004	4813	7320	GB:U09251_4	DNA gyrase subunit A (gyrA) {Mycoplasma genitalium}	99.8804	99.8804	MG004(1 - 2508 of 2508) GB:U09251(3282 - 5789 of 6140)
MG191	221571	225902	SP:P20796	attachment protein, MgPa operon (mgp) {Mycoplasma genitalium}	100	100	MG191(1 - 4332 of 4332) GB:M31431(1066 - 5397 of 8760)
MG192	225907	229062	SP:P22747	114 kDa protein, MgPa operon (mgp) {Mycoplasma genitalium}	100	100	MG192(1 - 3156 of 3156) GB:M31431(5402 - 8557 of 8760)
MG232	278904	279203	SP:P26908	ribosomal protein L21 (rpL21) {Bacillus subtilis}	37.8947	65.2632	MG232(1 - 300 of 300) GB:U02141(138 - 437 of 827)
MG233	279199	279495	GP:U02141_2	ribosomal protein L21 homolog {Mycoplasma genitalium}	100	100	MG233(1 - 297 of 297) GB:U02141(433 - 729 of 827)
MG287	348882	349133	SP:P04686	nodulation protein F (nodF) {Rhizobium leguminosarum}	34.9398	56.6265	MG287(1 - 252 of 252) GB:U01810(152 - 403 of 917)
MG417	521868	521473	SP:P07842	ribosomal protein S9 (rpS9) {Bacillus stearothermophilus}	51.9685	71.6535	MG417(1 - 396 of 396) GB:U01744(127 - 522 of 620)

Table 1(c)

UID	end5	end3	db_match	db_match name	per_sim	per_id	match_info
MG001	1026	1826	GB:U09251_1	DNA polymerase III beta subunit (dnaN) {Mycoplasma genitalium}	100	100	MG001(507 - 801 of 801) GB:U09251(1 - 295 of 6140)
MG005	7295	8545	GB:D26185_77	seryl-tRNA synthetase (serS) {Bacillus subtilis}	42.615	66.3438	MG005(1 - 377 of 1251) GB:U09251(5764 - 6140 of 6140)
MG005	7295	8545	GB:D26185_77	seryl-tRNA synthetase (serS) {Bacillus subtilis}	42.615	66.3438	MG005(16 - 337 of 1251) GB:U02210(1 - 322 of 322)
MG007	9157	9918	GB:D26185_83	DNA polymerase III subunit (dnaH) {Bacillus subtilis}	22.695	45.3901	MG007(762 - 711 of 762) GB:U02216(270 - 321 of 321)
MG008	9924	11249	GB:D26185_60	thiophene and furan oxidizer (tdhF) {Bacillus subtilis}	31.9101	59.7753	MG008(264 - 1 of 1326) GB:U02216(1 - 264 of 321)
MG011	13565	12705	-	-	-	-	MG011(473 - 767 of 861) GB:U02257(2 - 296 of 296)
MG014	15556	17424	SP:P27299	transport ATP-binding protein (msbA) {Escherichia coli}	28.0702	52.6316	MG014(1005 - 678 of 1869) GB:U02235(1 - 326 of 326)
MG018	21063	22343	SP:P32333	helicase (mot1) {Saccharomyces cerevisiae}	36.6972	60.0917	MG018(1281 - 1067 of 1281) GB:U01723(89 - 304 of 304)
MG018	21063	22343	SP:P32333	helicase (mot1) {Saccharomyces cerevisiae}	36.6972	60.0917	MG018(409 - 105 of 1281) GB:U02179(1 - 305 of 305)
MG018	21063	22343	SP:P32333	helicase (mot1) {Saccharomyces cerevisiae}	36.6972	60.0917	MG018(592 - 896 of 1281) GB:U01757(1 - 305 of 305)

MG019	22388	23554	SP:P35514	heat shock protein (dnaJ) {Lactococcus lactis}	33.9779	51.105	MG019(44 - 1 of 1167) GB:U01723(1 - 44 of 304)
MG020	23541	24464	GB:Z25461_2	proline iminopeptidase (pip) {Neisseria gonorrhoeae}	37.5439	55.7895	MG020(723 - 924 of 924) GB:U02229(1 - 202 of 333)
MG021	24467	26002	GB:D26185_101	methionyl-tRNA synthetase (metS) {Bacillus subtilis}	37.5494	58.8933	MG021(1 - 129 of 1536) GB:U02229(205 - 333 of 333)
MG021	24467	26002	GB:D26185_101	methionyl-tRNA synthetase (metS) {Bacillus subtilis}	37.5494	58.8933	MG021(1318 - 1527 of 1536) GB:X61513(1 - 209 of 209)
MG022	26035	26469	GB:M21677_1	RNA polymerase delta subunit (rpoE) {Bacillus subtilis}	28.6765	49.2647	MG022(254 - 1 of 435) GB:U01721(1 - 254 of 299)
MG025	28651	29544	GP:Z47767_4	TrsB {Yersinia enterocolitica}	27.551	54.0816	MG025(514 - 894 of 894) GB:U02253(1 - 381 of 649)
MG026	29551	30120	GB:U14003_62	elongation factor P (efp) {Escherichia coli}	26.3804	47.2393	MG026(1 - 262 of 570) GB:U02253(388 - 649 of 649)
MG029	31702	31145	GB:L19300_1	hypothetical protein (GB:L19300_1) {Staphylococcus aureus}	27.027	45.045	MG029(1 - 93 of 558) GB:U01773(210 - 302 of 302)
MG030	32324	31707	GB:Z27121_3	uracil phosphoribosyltransferase (upp) {Mycoplasma hominis}	44.9275	66.6667	MG030(414 - 618 of 618) GB:U01773(1 - 205 of 302)
MG031	36713	32361	GB:U06833_1	DNA polymerase III (polC) {Mycoplasma pulmonis}	38.0303	59.3182	MG031(1473 - 1701 of 4353) GB:U01807(1 - 229 of 229)
MG031	36713	32361	GB:U06833_1	DNA polymerase III (polC) {Mycoplasma pulmonis}	38.0303	59.3182	MG031(2923 - 3309 of 4353) GB:U01712(1 - 387 of 387)
MG031	36713	32361	GB:U06833_1	DNA polymerase III (polC) {Mycoplasma pulmonis}	38.0303	59.3182	MG031(3330 - 3676 of 4353) GB:U02208(1 - 347 of 347)

MG036	41777	43426	SP:P36419	aspartyl-tRNA synthetase (aspS) {Thermus aquaticus}	40.8582	62.8731	MG036(1115 - 1650 of 1650) GB:U01814(1 - 532 of 1006)
MG036	41777	43426	SP:P36419	aspartyl-tRNA synthetase (aspS) {Thermus aquaticus}	40.8582	62.8731	MG036(1407 - 1638 of 1650) GB:X61511(1 - 232 of 232)
MG036	41777	43426	SP:P36419	aspartyl-tRNA synthetase (aspS) {Thermus aquaticus}	40.8582	62.8731	MG036(1412 - 1160 of 1650) GB:X61523(1 - 252 of 252)
MG037	43402	44751	GP:U02020_1	pre-B cell enhancing factor (PBEF) {Homo sapiens}	34.3164	52.2788	MG037(1 - 500 of 1350) GB:U01814(508 - 1006 of 1006)
MG040	47581	49353	SP:P29724	membrane lipoprotein (tmpC) {Treponema pallidum}	30.8594	48.0469	MG040(1341 - 1552 of 1773) GB:U02125(1 - 212 of 212)
MG045	53205	54653	-	-	-	-	MG045(381 - 4 of 1449) GB:U02166(1 - 378 of 378)
MG047	55589	56737	SP:P30869	S-adenosylmethionine synthetase 2 (metX) {Escherichia coli}	43.6111	60.5556	MG047(787 - 1070 of 1149) GB:U02123(1 - 284 of 284)
MG051	59741	61003	GB:L13289_3	thymidine phosphorylase (deoA) {Mycoplasma pirum}	52.7316	73.6342	MG051(1161 - 1263 of 1263) GB:U02191(1 - 103 of 183)
MG052	61015	61404	GB:L13289_4	cytidine deaminase (cdd) {Mycoplasma pirum}	38.2114	64.2276	MG052(1 - 69 of 390) GB:U02191(115 - 183 of 183)
MG052	61015	61404	GB:L13289_4	cytidine deaminase (cdd) {Mycoplasma pirum}	38.2114	64.2276	MG052(320 - 390 of 390) GB:U02108(1 - 71 of 212)
MG053	61407	63056	GB:L13289_5	phosphomannomutase (cpsG) {Mycoplasma pirum}	38.7868	58.0882	MG053(1 - 140 of 1650) GB:U02108(74 - 212 of 212)
MG054	63986	63039	GB:D13303_4	transcription antitermination factor (nusG) {Bacillus subtilis}	30.8571	51.4286	MG054(688 - 44 of 948) GB:U01710(1 - 645 of 645)

MG054	63986	63039	GB:D13303_4	transcription antitermination factor (nusG) {Bacillus subtilis}	30.8571	51.4286	MG054(948 - 719 of 948) GB:U02236(45 - 274 of 276)
MG055	64361	63993	-	-	-	-	MG055(1 - 326 of 369) GB:U02240(23 - 348 of 348)
MG058	67121	66231	GB:D26185_114	phosphoribosylpyrophosphate synthetase (prs) {Bacillus subtilis}	44.4089	63.5783	MG058(72 - 1 of 891) GB:U01693(1 - 72 of 350)
MG059	67644	67210	GB:D12501_1	small protein (smpB) {Escherichia coli}	32.5581	62.0155	MG059(435 - 247 of 435) GB:U01693(161 - 350 of 350)
MG060	67651	68541	SP:P26401	lipopolysaccharide biosynthesis protein (rfbV) {Salmonella typhimurium}	36.0656	59.8361	MG060(723 - 396 of 891) GB:U02262(1 - 328 of 328)
MG061	69908	68526	GB:M89480_4	hexosephosphate transport protein (uhpT) {Salmonella typhimurium}	30.9091	57.2727	MG061(1273 - 613 of 1383) GB:U01705(1 - 661 of 661)
MG062	70531	72570	SP:P20966	fructose-permease IIBC component (fruA) {Escherichia coli}	42.723	60.5634	MG062(439 - 761 of 2040) GB:U02138(1 - 323 of 323)
MG063	72668	73432	SP:P23539	1-phosphofructokinase (fruK) {Escherichia coli}	26.3158	51.5038	MG063(363 - 626 of 765) GB:U01777(1 - 264 of 264)
MG065	77686	79083	GB:X75422_1	heterocyst maturation protein (devA) {Anabaena sp.}	35.2941	59.7285	MG065(1398 - 1176 of 1398) GB:U02154(133 - 354 of 354)
MG066	79090	81033	SP:P27302	transketolase 1 (TK 1) (tktA) {Escherichia coli}	32.5617	54.9383	MG066(126 - 1 of 1944) GB:U02154(1 - 126 of 354)
MG068	82621	84042	-	-	-	-	MG068(1244 - 919 of 1422) GB:U02162(1 - 326 of 326)
MG069	88228	90951	SP:P20166	phosphotransferase enzyme II, ABC component (ptsG) {Bacillus subtilis}	43.1596	61.0749	MG069(1127 - 849 of 2724) GB:U02207(1 - 279 of 279)

MG071	91924	94545	SP:P37278	cation-transporting ATPase (pacL) {Synechococcus sp.}	34.3897	57.277	MG071(1470 - 1209 of 2622) GB:X61532(1 - 262 of 262)
MG072	94535	96952	GB:D10279_2	preprotein translocase (secA) {Bacillus subtilis}	43.6601	66.7974	MG072(2269 - 2418 of 2418) GB:U01743(1 - 150 of 365)
MG073	96933	98900	SP:P07025	excinuclease ABC subunit B (uvrB) {Escherichia coli}	47.9751	67.2897	MG073(1 - 235 of 1968) GB:U01743(131 - 365 of 365)
MG073	96933	98900	SP:P07025	excinuclease ABC subunit B (uvrB) {Escherichia coli}	47.9751	67.2897	MG073(1584 - 1240 of 1968) GB:U01698(1 - 345 of 345)
MG073	96933	98900	SP:P07025	excinuclease ABC subunit B (uvrB) {Escherichia coli}	47.9751	67.2897	MG073(305 - 694 of 1968) GB:U02119(1 - 391 of 391)
MG074	98906	99316	-	-	-	-	MG074(369 - 411 of 411) GB:U01715(1 - 43 of 576)
MG075	99383	102454	-	-	-	-	MG075(1 - 467 of 3072) GB:U01715(110 - 576 of 576)
MG075	99383	102454	-	-	-	-	MG075(1206 - 804 of 3072) GB:U02251(1 - 403 of 403)
MG075	99383	102454	-	-	-	-	MG075(1927 - 2210 of 3072) GB:U01749(1 - 284 of 284)
MG075	99383	102454	-	-	-	-	MG075(2841 - 2422 of 3072) GB:U01775(1 - 420 of 420)
MG080	106660	109203	SP:P18766	oligopeptide transport ATP-binding protein (amiF) {Streptococcus pneumoniae}	46.6403	67.1937	MG080(2268 - 1954 of 2544) GB:U02129(1 - 315 of 315)
MG080	106660	109203	SP:P18766	oligopeptide transport ATP-binding protein (amiF) {Streptococcus pneumoniae}	46.6403	67.1937	MG080(951 - 646 of 2544) GB:U01758(1 - 306 of 306)

MG082	109675	110352	SP:P04447	ribosomal protein L1 (rpL1) {Bacillus stearothermophilus}	48.1982	67.5676	MG082(446 - 170 of 678) GB:U02113(1 - 278 of 278)
MG083	110355	110921	GB:L32144_1	peptidyl-tRNA hydrolase homolog (pth) {Borrelia burgdorferi}	38.2166	57.3248	MG083(567 - 220 of 567) GB:U02185(26 - 373 of 373)
MG084	110917	111786	SP:P37563	hypothetical protein (SP:P37563) {Bacillus subtilis}	28.125	46.3542	MG084(30 - 1 of 870) GB:U02185(1 - 30 of 373)
MG084	110917	111786	SP:P37563	hypothetical protein (SP:P37563) {Bacillus subtilis}	28.125	46.3542	MG084(794 - 870 of 870) GB:U01783(1 - 77 of 269)
MG087	113895	114311	SP:P09901	ribosomal protein S12 (rpS12) {Bacillus stearothermophilus}	75.3731	82.0896	MG087(417 - 349 of 417) GB:U02212(326 - 394 of 394)
MG088	114331	114795	SP:P22744	ribosomal protein S7 (rpS7) {Bacillus stearothermophilus}	64.9351	81.1688	MG088(305 - 1 of 465) GB:U02212(2 - 306 of 394)
MG089	114808	116871	SP:P13551	elongation factor G (fus) {Thermus aquaticus}	59.2105	78.0702	MG089(1878 - 1540 of 2064) GB:U02180(1 - 339 of 340)
MG089	114808	116871	SP:P13551	elongation factor G (fus) {Thermus aquaticus}	59.2105	78.0702	MG089(1885 - 2064 of 2064) GB:U02136(1 - 180 of 410)
MG089	114808	116871	SP:P13551	elongation factor G (fus) {Thermus aquaticus}	59.2105	78.0702	MG089(687 - 1374 of 2064) GB:U01722(1 - 688 of 688)
MG090	116926	117549	SP:P02358	ribosomal protein S6 (rpS6) {Escherichia coli}	23.8636	44.3182	MG090(1 - 176 of 624) GB:U02136(235 - 410 of 410)
MG094	118847	120184	SP:P03005	replicative DNA helicase (dnaB) {Escherichia coli}	33.105	55.0228	MG094(1068 - 731 of 1338) GB:U01803(1 - 336 of 336)
MG094	118847	120184	SP:P03005	replicative DNA helicase (dnaB) {Escherichia coli}	33.105	55.0228	MG094(228 - 1 of 1338) GB:U02158(1 - 228 of 301)

MG095	120191	121384	-	-	-	-	MG095(355 - 759 of 1194) GB:U01787(1 - 403 of 403)
MG096	121939	123519	-	-	-	-	MG096(1 - 309 of 1581) GB:U01713(58 - 366 of 366)
MG096	121939	123519	-	-	-	-	MG096(361 - 531 of 1581) GB:U01762(1 - 171 of 171)
MG097	123579	124313	GB:D13169_3	uracil DNA glycosylase (ung) {Escherichia coli}	32.5688	51.8349	MG097(220 - 694 of 735) GB:U02201(1 - 475 of 475)
MG098	124416	125846	GP:M74170_2	p48 eggshell protein (p48) {Schistosoma mansoni}	23.0769	47.9853	MG098(1260 - 831 of 1431) GB:U01782(1 - 431 of 431)
MG098	124416	125846	GP:M74170_2	p48 eggshell protein (p48) {Schistosoma mansoni}	23.0769	47.9853	MG098(134 - 467 of 1431) GB:U01701(1 - 334 of 334)
MG100	127278	128708	GP:L22072_1	PET112 protein {Saccharomyces cerevisiae}	30.8696	54.1304	MG100(533 - 238 of 1431) GB:U01799(1 - 296 of 296)
MG101	128686	129351	-	-	-	-	MG101(89 - 398 of 666) GB:U02103(1 - 309 of 309)
MG102	129347	130291	GB:J03762_1	thioredoxin reductase (trxB) {Escherichia coli}	38.5906	59.396	MG102(45 - 367 of 945) GB:U02197(1 - 322 of 322)
MG103	130284	131123	-	-	-	-	MG103(623 - 256 of 840) GB:U02170(1 - 368 of 369)
MG104	131384	133558	GB:U14003_91	virulence associated protein homolog (vacB) {Escherichia coli}	29.2335	52.2282	MG104(215 - 491 of 2175) GB:U01795(1 - 277 of 277)
MG108	135337	136116	SP:P35182	protein phosphatase 2C homolog (ptc1) {Saccharomyces cerevisiae}	27.5362	52.1739	MG108(780 - 598 of 780) GB:U02111(33 - 215 of 215)

MG109	136179	137264	PIR:S36944	protein serine/threonine kinase {Arabidopsis thaliana}	33.7398	52.0325	MG109(425 - 786 of 1086) GB:U01720(1 - 362 of 362)
MG109	136179	137264	PIR:S36944	protein serine/threonine kinase {Arabidopsis thaliana}	33.7398	52.0325	MG109(781 - 1084 of 1086) GB:U01748(1 - 303 of 303)
MG110	137380	138087	GB:U14003_76	hypothetical protein (GB:U14003_76) {Escherichia coli}	28.5714	54.1126	MG110(140 - 242 of 708) GB:X61518(1 - 102 of 102)
MG110	137380	138087	GB:U14003_76	hypothetical protein (GB:U14003_76) {Escherichia coli}	28.5714	54.1126	MG110(670 - 378 of 708) GB:U01714(1 - 293 of 293)
MG111	138105	139403	SP:P13376	phosphoglucose isomerase B (pgiB) {Bacillus stearothermophilus}	34.8235	53.6471	MG111(1 - 98 of 1299) GB:U01747(38 - 135 of 135)
MG112	139396	140022	GB:M64173_3	D-ribulose-5-phosphate 3 epimerase (cfxEc) {Alcaligenes eutrophus}	33.1361	53.8462	MG112(207 - 473 of 627) GB:U02181(1 - 267 of 267)
MG113	140039	141406	GB:M33145_1	asparaginyl-tRNA synthetase (asnS) {Escherichia coli}	41.4579	64.2369	MG113(1231 - 941 of 1368) GB:U01692(1 - 291 of 291)
MG115	142314	142550	SP:P31131	hypothetical protein (SP:P31131) {Escherichia coli}	32.6087	50	MG115(198 - 237 of 237) GB:U02127(1 - 40 of 234)
MG116	142562	143314	-	-	-	-	MG116(1 - 183 of 753) GB:U02127(52 - 234 of 234)
MG119	144972	146663	GB:M59444_2	methylgalactoside permease ATP-binding protein (mglA) {Escherichia coli}	33.1984	57.6923	MG119(1660 - 1692 of 1692) GB:U02147(1 - 33 of 301)
MG119	144972	146663	GB:M59444_2	methylgalactoside permease ATP-binding protein (mglA) {Escherichia coli}	33.1984	57.6923	MG119(192 - 1 of 1692) GB:U02149(1 - 192 of 681)
MG120	146673	148232	SP:P36948	ribose transport system permease protein (rbsC) {Bacillus subtilis}	27.4809	51.9084	MG120(1 - 259 of 1560) GB:U02147(43 - 301 of 301)

MG122	149198	151324	GB:L27797_2	DNA topoisomerase I (topA) {Bacillus subtilis}	38.9222	59.7305	MG122(1193 - 1443 of 2127) GB:U02134(1 - 251 of 251)
MG122	149198	151324	GB:L27797_2	DNA topoisomerase I (topA) {Bacillus subtilis}	38.9222	59.7305	MG122(1578 - 1971 of 2127) GB:U02242(1 - 394 of 394)
MG123	151305	152717	GB:M91593_1	hypothetical protein (GB:M91593_1) {Mycoplasma mycoides}	23.9837	50.4065	MG123(1413 - 1236 of 1413) GB:U01796(114 - 291 of 291)
MG124	152767	153072	GB:J03294_1	thioredoxin (trx) {Bacillus subtilis}	36.0825	65.9794	MG124(64 - 1 of 306) GB:U01796(1 - 64 of 291)
MG133	159669	158986	-	-	-	-	MG133(1 - 110 of 684) GB:U02144(237 - 345 of 345)
MG133	159669	158986	-	-	-	-	MG133(435 - 673 of 684) GB:X61537(1 - 238 of 238)
MG134	159797	160096	GB:M38777_3	hypothetical protein (GB:M38777_3) {Escherichia coli}	28.5714	57.1429	MG134(109 - 1 of 300) GB:U02144(1 - 109 of 345)
MG135	160913	160074	PIR:E22845	hypothetical protein 4 (GP:Z33006_1) {Trypanosoma brucei}	30.7692	55.9441	MG135(485 - 782 of 840) GB:U02114(1 - 298 of 298)
MG138	163590	165383	GB:K00426_1	GTP-binding membrane protein (lepA) {Escherichia coli}	47.5465	70.5584	MG138(1237 - 938 of 1794) GB:U02133(2 - 301 of 301)
MG138	163590	165383	GB:K00426_1	GTP-binding membrane protein (lepA) {Escherichia coli}	47.5465	70.5584	MG138(1318 - 1794 of 1794) GB:U01745(1 - 477 of 524)
MG138	163590	165383	GB:K00426_1	GTP-binding membrane protein (lepA) {Escherichia coli}	47.5465	70.5584	MG138(323 - 591 of 1794) GB:X61521(1 - 269 of 269)
MG140	175807	179145	-	-	-	-	MG140(1 - 41 of 3339) GB:U02110(178 - 218 of 218)

MG140	175807	179145	-	-	-	-	MG140(2727 - 2429 of 3339) GB:U01730(1 - 297 of 297)
MG140	175807	179145	-	-	-	-	MG140(3302 - 2994 of 3339) GB:U02156(1 - 308 of 308)
MG140	175807	179145	-	-	-	-	MG140(382 - 834 of 3339) GB:U01729(1 - 454 of 454)
MG140	175807	179145	-	-	-	-	MG140(834 - 616 of 3339) GB:X61512(1 - 220 of 220)
MG140	175807	179145	-	-	-	-	MG140(880 - 1182 of 3339) GB:U01742(1 - 303 of 303)
MG141	179153	180745	SP:P32727	N-utilization substance protein A homolog (nusA) {Bacillus subtilis}	30.8743	53.8251	MG141(223 - 871 of 1593) GB:U01778(1 - 652 of 652)
MG142	181007	182863	GB:M34836_1	protein synthesis initiation factor 2 (infB) {Bacillus subtilis}	46.0292	64.6677	MG142(265 - 393 of 1857) GB:U01765(1 - 129 of 129)
MG144	183216	184052	-	-	-	-	MG144(190 - 420 of 837) GB:U02121(1 - 231 of 231)
MG146	184877	186148	GB:X73141_2	hemolysin (tlyC) {Serpulina hyodysenteriae}	26.2712	52.1186	MG146(1272 - 1174 of 1272) GB:U02223(19 - 117 of 117)
MG149	188609	189451	-	-	-	-	MG149(843 - 765 of 843) GB:U02135(182 - 260 of 260)
MG151	190372	191142	SP:P10134	ribosomal protein L3 (rpL3) {Mycoplasma capricolum}	42.5926	61.5741	MG151(528 - 1 of 771) GB:U02153(1 - 527 of 543)
MG168	198519	199151	GB:M57621_1	ribosomal protein S5 (rpS5) {Bacillus stearothermophilus}	55.9748	72.327	MG168(505 - 633 of 633) GB:U01726(1 - 129 of 260)

MG175	202762	203133	GB:M26414_3	ribosomal protein S13 (rpS13) {Bacillus subtilis}	63.3333	82.5	MG175(22 - 372 of 372) GB:U01733(1 - 351 of 600)
MG176	203136	203528	GB:X02543_2	ribosomal protein S11 (rpS11) {Escherichia coli}	47.7876	69.9115	MG176(1 - 247 of 393) GB:U01733(354 - 600 of 600)
MG180	205682	206593	GB:M61017_1	membrane transport protein (glnQ) {Bacillus stearothermophilus}	37.3832	63.0841	MG180(249 - 1 of 912) GB:U01754(1 - 248 of 265)
MG180	205682	206593	GB:M61017_1	membrane transport protein (glnQ) {Bacillus stearothermophilus}	37.3832	63.0841	MG180(912 - 784 of 912) GB:U01750(167 - 295 of 295)
MG181	206589	207848	-	-	-	-	MG181(171 - 1 of 1260) GB:U01750(1 - 171 of 295)
MG182	207844	208575	SP:P07649	pseudouridylate synthase I (hisT) {Escherichia coli}	27.0042	45.1477	MG182(1 - 308 of 732) GB:U02176(70 - 377 of 377)
MG182	207844	208575	SP:P07649	pseudouridylate synthase I (hisT) {Escherichia coli}	27.0042	45.1477	MG182(732 - 383 of 732) GB:U02100(31 - 380 of 380)
MG183	208568	210388	GB:Z32522_1	oligoendopeptidase F (pepF) {Lactococcus lactis}	30	50.6667	MG183(27 - 335 of 1821) GB:U02198(1 - 309 of 309)
MG183	208568	210388	GB:Z32522_1	oligoendopeptidase F (pepF) {Lactococcus lactis}	30	50.6667	MG183(38 - 1 of 1821) GB:U02100(1 - 38 of 380)
MG184	210392	211342	GB:M97479_2	methyltransferase (ssoIM) {Shigella sonnei}	42.5249	67.4419	MG184(520 - 719 of 951) GB:U02115(1 - 200 of 201)
MG190	220479	221561	PIR:JS0068	29 kDa protein, MgPa operon (mgp) {Mycoplasma genitalium}	62.0833	82.0833	MG190(28 - 1083 of 1083) GB:M31431(1 - 1056 of 8760)
MG194	232007	233029	GB:V00291_5	phenylalanyl-tRNA synthetase beta-subunit (pheS) {Escherichia coli}	35.0769	56.3077	MG194(194 - 359 of 1023) GB:U02120(1 - 166 of 166)

MG195	233036	235453	SP:P17922	phenylalanyl-tRNA synthetase beta chain (pheT) {Bacillus subtilis}	25.4597	49.0806	MG195(2044 - 2396 of 2418) GB:U02173(1 - 353 of 353)
MG200	237346	239148	GB:L36455_1	heat shock protein (dnaJ) {Coxiella burnetii}	33.5938	51.5625	MG200(842 - 1227 of 1803) GB:U02163(2 - 387 of 387)
MG203	240322	242220	GB:U25549_1	topoisomerase IV subunit B (parE) {Mycoplasma genitalium}	100	100	MG203(1216 - 1899 of 1899) GB:U25549(1 - 684 of 2124)
MG204	242223	244565	GB:U25549_2	topoisomerase IV subunit A (parC) {Mycoplasma genitalium}	99.7912	99.7912	MG204(1 - 1438 of 2343) GB:U25549(687 - 2124 of 2124)
MG204	242223	244565	GB:U25549_2	topoisomerase IV subunit A (parC) {Mycoplasma genitalium}	99.7912	99.7912	MG204(1950 - 1641 of 2343) GB:U02155(1 - 308 of 308)
MG206	246127	247422	SP:P14951	excinuclease ABC subunit C (uvrC)	28.0872	51.0896	MG206(738 - 399 of 1296) GB:U02182(1 - 341 of 341)
MG208	248492	247905	-	-	-	-	MG208(585 - 162 of 588) GB:U01785(1 - 423 of 423)
MG209	249402	248479	SP:P23851	hypothetical protein (SP:P23851) {Escherichia coli}	30.4498	55.0173	MG209(730 - 372 of 924) GB:U02214(1 - 359 of 359)
MG210	249947	249405	GB:M83994_1	prolipoprotein signal peptidase (lsp) {Staphylococcus aureus}	32.3944	52.1127	MG210(1 - 116 of 543) GB:U01759(196 - 311 of 311)
MG212	251780	252583	GB:L32861_1	1-acyl-sn-glycerol-3-phosphate acetyltransferase (plsC) {Borrelia burgdorferi}	32.1429	60.7143	MG212(7 - 315 of 804) GB:U02160(5 - 313 of 313)
MG216	255594	257117	GB:L07920_2	pyruvate kinase (pyk) {Lactococcus lactis}	35.3319	57.6017	MG216(1118 - 790 of 1524) GB:U01798(1 - 329 of 329)
MG218	259176	264590	PIR:S37536	no score generated - score shown is bogus	-1	-1	MG218(1669 - 1977 of 5415) GB:U02165(1 - 309 of 309)

MG221	266626	267087	SP:P22186	hypothetical protein (SP:P22186) {Escherichia coli}	28.8732	56.338	MG221(337 - 49 of 462) GB:U02195(1 - 290 of 290)
MG225	270404	271870	GB:U14003_71	hypothetical protein (GB:U14003_71) {Escherichia coli}	21.9565	48.0435	MG225(1467 - 1409 of 1467) GB:U02264(289 - 347 of 347)
MG226	271938	273314	GB:D26562_11	aromatic amino acid transport protein (aroP) {Escherichia coli}	24.5902	47.2131	MG226(221 - 1 of 1377) GB:U02264(1 - 221 of 347)
MG227	273789	274649	SP:P13954	thymidylate synthase (thyA) {Staphylococcus aureus}	56.5972	75.3472	MG227(577 - 861 of 861) GB:U01718(1 - 285 of 439)
MG228	274652	275131	GB:X60681_1	dihydrofolate reductase (dhfr) {Lactococcus lactis}	33.1288	59.5092	MG228(480 - 385 of 480) GB:U02137(174 - 269 of 269)
MG229	275140	276159	SP:P17424	ribonucleotide reductase 2 (nrdF) {Salmonella typhimurium}	50	70.0637	MG229(1020 - 697 of 1020) GB:U01739(22 - 344 of 344)
MG231	276646	278808	GB:X73226_1	ribonucleoside-diphosphate reductase (nrdE) {Salmonella typhimurium}	54.1193	73.1534	MG231(2122 - 2163 of 2163) GB:U02141(1 - 42 of 827)
MG237	281078	281959	-	-	-	-	MG237(647 - 882 of 882) GB:U01774(1 - 236 of 289)
MG238	281992	283323	GB:M34066_1	trigger factor (tig) {Escherichia coli}	24.6193	47.9695	MG238(420 - 648 of 1332) GB:U01772(1 - 229 of 229)
MG239	283395	285779	SP:P37945	ATP-dependent protease (lon) {Bacillus subtilis}	43.6268	65.8344	MG239(1818 - 1449 of 2385) GB:U02148(1 - 370 of 370)
MG240	286657	285782	GB:M91593_1	hypothetical protein (GB:M91593_1) {Mycoplasma mycoides}	27.8195	53.3835	MG240(876 - 598 of 876) GB:U01734(27 - 305 of 305)
MG242	288752	290641	-	-	-	-	MG242(886 - 543 of 1890) GB:U02194(1 - 344 of 344)

MG244	291332	293440	GB:M99049_1	DNA helicase II (mutB1) {Haemophilus influenzae}	36.0078	55.9687	MG244(829 - 1035 of 2109) GB:X61517(1 - 207 of 207)
MG249	297604	296114	SP:P33656	RNA polymerase sigma-A factor (sigA) {Clostridium acetobutylicum}	43.6842	66.0526	MG249(970 - 666 of 1491) GB:X61535(1 - 306 of 306)
MG250	299472	297652	GB:M10040_1	DNA primase (dnaE) {Bacillus subtilis}	27.2727	52.2078	MG250(1530 - 1821 of 1821) GB:U01771(1 - 292 of 572)
MG250	299472	297652	GB:M10040_1	DNA primase (dnaE) {Bacillus subtilis}	27.2727	52.2078	MG250(648 - 231 of 1821) GB:U02146(1 - 418 of 418)
MG254	304823	302847	GB:M24278_1	DNA ligase (lig) {Escherichia coli}	38.2263	59.3272	MG254(1429 - 1722 of 1977) GB:U02152(1 - 294 of 294)
MG254	304823	302847	GB:M24278_1	DNA ligase (lig) {Escherichia coli}	38.2263	59.3272	MG254(37 - 367 of 1977) GB:U01761(1 - 330 of 330)
MG255	304999	306093	-	-	-	-	MG255(726 - 1095 of 1095) GB:U02164(1 - 370 of 370)
MG255	304999	306093	-	-	-	-	MG255(729 - 400 of 1095) GB:U02174(1 - 333 of 333)
MG261	315699	318320	GB:M19334_4	DNA polymerase III alpha subunit (dnaE) {Escherichia coli}	31.9115	55.7662	MG261(2442 - 2159 of 2622) GB:U01738(1 - 284 of 284)
MG263	320175	321047	GB:L10328_61	hypothetical protein (GB:L10328_61) {Escherichia coli}	27.8008	47.7178	MG263(828 - 489 of 873) GB:U01764(1 - 340 of 340)
MG266	324809	322434	GB:M88581_1	leucyl-tRNA synthetase (leuS) {Bacillus stearothermophilus}	43.401	64.2132	MG266(78 - 287 of 2376) GB:U01780(1 - 210 of 210)
MG266	324809	322434	GB:M88581_1	leucyl-tRNA synthetase (leuS) {Bacillus stearothermophilus}	43.401	64.2132	MG266(957 - 622 of 2376) GB:U02167(1 - 336 of 336)

MG269	327050	326031	GB:D90354_1	surface protein antigen precursor (pag) {Streptococcus sobrinus}	25.5144	47.3251	MG269(239 - 1 of 1020) GB:U02215(1 - 239 of 366)
MG271	329826	328456	SP:P11959	dihydrolipoamide dehydrogenase (pdhD) {Bacillus stearothermophilus}	38.3592	62.306	MG271(914 - 1214 of 1371) GB:U01784(1 - 301 of 301)
MG275	334772	333339	SP:P37061	NADH oxidase (nox) {Enterococcus faecalis}	39.229	62.1315	MG275(81 - 1 of 1434) GB:U01786(4 - 84 of 280)
MG276	335397	334858	GB:M14040_1	adenine phosphoribosyltransferase (apt) {Escherichia coli}	34.3373	58.4337	MG276(540 - 430 of 540) GB:U01786(170 - 280 of 280)
MG278	338366	340525	GB:X72832_5	stringent response-like protein (rel) {Streptococcus equisimilis}	29.1339	55.1181	MG278(391 - 697 of 2160) GB:U01770(1 - 308 of 308)
MG281	343702	342035	-	-	-	-	MG281(748 - 1051 of 1668) GB:U01706(1 - 303 of 303)
MG282	344849	344367	SP:P27640	transcription elongation factor (greA) {Rickettsia prowazekii}	40.146	65.6934	MG282(483 - 356 of 483) GB:U02104(187 - 314 of 314)
MG283	345181	346629	GB:M97858_1	prolyl-tRNA synthetase (proS) {Escherichia coli}	22.6562	46.0938	MG283(839 - 1183 of 1449) GB:U02205(1 - 346 of 346)
MG285	347214	348254	-	-	-	-	MG285(315 - 493 of 1041) GB:U02266(1 - 180 of 180)
MG289	354023	355126	SP:P15363	high affinity transport system protein P37 (P37) {Mycoplasma hyorhinis}	35.7798	58.4098	MG289(105 - 1 of 1104) GB:U02132(1 - 105 of 571)
MG291	355846	357474	SP:P15362	transport system permease protein P69 (P69) {Mycoplasma hyorhinis}	27.9159	54.8757	MG291(1216 - 1629 of 1629) GB:U01768(1 - 415 of 705)
MG291	355846	357474	SP:P15362	transport system permease protein P69 (P69) {Mycoplasma hyorhinis}	27.9159	54.8757	MG291(279 - 1 of 1629) GB:U02171(1 - 279 of 346)

MG293	361384	360653	SP:P37965	glycerophosphoryl diester phosphodiesterase (glpQ) {Bacillus subtilis}	30.3965	55.9471	MG293(357 - 41 of 732) GB:U02118(1 - 317 of 317)
MG294	362801	361380	GB:L19201_18	hypothetical protein (GB:L19201_18) {Escherichia coli}	23.1013	46.2025	MG294(256 - 592 of 1422) GB:U02243(1 - 337 of 337)
MG297	365574	364537	GB:U00039_18	cell division protein (ftsY) {Escherichia coli}	36.1371	57.9439	MG297(1 - 57 of 1038) GB:U02177(215 - 271 of 271)
MG298	368529	365584	GB:M34956_1	115 kDa protein (p115) {Mycoplasma hyorhinis}	33.4059	57.5626	MG298(2743 - 2946 of 2946) GB:U02177(1 - 205 of 271)
MG300	370962	369715	SP:P36204	phosphoglycerate kinase (pgk) {Thermotoga maritima}	51.2887	70.6186	MG300(1 - 167 of 1248) GB:U02178(167 - 333 of 333)
MG300	370962	369715	SP:P36204	phosphoglycerate kinase (pgk) {Thermotoga maritima}	51.2887	70.6186	MG300(935 - 609 of 1248) GB:U02226(1 - 326 of 326)
MG300	370962	369715	SP:P36204	phosphoglycerate kinase (pgk) {Thermotoga maritima}	51.2887	70.6186	MG300(939 - 1243 of 1248) GB:U02234(1 - 305 of 305)
MG301	371962	370952	GB:X72219_1	glyceraldehyde-3-phosphate dehydrogenase (gap) {Clostridium pasteurianum}	56.0606	73.0303	MG301(244 - 1 of 1011) GB:U02213(1 - 244 of 364)
MG301	371962	370952	GB:X72219_1	glyceraldehyde-3-phosphate dehydrogenase (gap) {Clostridium pasteurianum}	56.0606	73.0303	MG301(835 - 1011 of 1011) GB:U02178(1 - 177 of 333)
MG302	372946	371996	-	-	-	-	MG302(951 - 865 of 951) GB:U02213(278 - 364 of 364)
MG305	376705	374921	GB:D30690_3	heat shock protein 70 (hsp70) {Staphylococcus aureus}	57.4359	75.8974	MG305(1382 - 1055 of 1785) GB:U02204(1 - 327 of 327)
MG307	381507	377977	-	-	-	-	MG307(3175 - 2042 of 3531) GB:U01767(1 - 1134 of 1134)

MG308	382724	381495	SP:P23304	ATP-dependent RNA helicase (deaD) {Escherichia coli}	23.0986	48.169	MG308(1 - 89 of 1230) GB:U02200(276 - 364 of 364)
MG309	386408	382734	-	-	-	-	MG309(3410 - 3675 of 3675) GB:U02200(1 - 266 of 364)
MG312	391334	387918	GB:U11381_1	cytadherence-accessory protein (hmw1) {Mycoplasma pneumoniae}	39.3235	60.6765	MG312(2541 - 2160 of 3417) GB:U02261(1 - 382 of 382)
MG314	393633	392305	GP:L38997_4	hypothetical protein (GP:L38997_4) {Mycoplasma pneumoniae}	51.4477	71.4922	MG314(514 - 206 of 1329) GB:U02151(1 - 309 of 309)
MG317	397423	395627	GB:M82965_1	cytadherence-accessory protein (hmw3) {Mycoplasma pneumoniae}	41.1458	59.8958	MG317(1329 - 1542 of 1797) GB:U02267(1 - 214 of 214)
MG317	397423	395627	GB:M82965_1	cytadherence-accessory protein (hmw3) {Mycoplasma pneumoniae}	41.1458	59.8958	MG317(509 - 169 of 1797) GB:U02224(1 - 341 of 341)
MG317	397423	395627	GB:M82965_1	cytadherence-accessory protein (hmw3) {Mycoplasma pneumoniae}	41.1458	59.8958	MG317(73 - 1 of 1797) GB:U01716(1 - 73 of 325)
MG318	398280	397441	GB:J04151_1	fibronectin-binding protein (fnbA) {Staphylococcus aureus}	24.6154	43.0769	MG318(840 - 604 of 840) GB:U01716(91 - 325 of 325)
MG319	398833	398300	-	-	-	-	MG319(423 - 1 of 534) GB:U01769(1 - 426 of 541)
MG320	399797	398940	-	-	-	-	MG320(371 - 781 of 858) GB:U01700(1 - 410 of 410)
MG324	408792	407731	GB:D00398_1	aminopeptidase P (pepP) {Escherichia coli}	30.531	54.4248	MG324(883 - 1062 of 1062) GB:U01717(1 - 181 of 223)
MG324	408792	407731	GB:D00398_1	aminopeptidase P (pepP) {Escherichia coli}	30.531	54.4248	MG324(889 - 1062 of 1062) GB:U01755(2 - 175 of 217)

MG327	410676	409873	SP:P26174	magnesium-chelatase 30 kDa subunit (bchO) {Rhodobacter capsulatus}	26.7281	51.1521	MG327(782 - 533 of 804) GB:U02232(1 - 250 of 250)
MG328	412933	410666	GB:X62467_1	protein V (fcrV) {Streptococcus sp.}	27.5434	48.3871	MG328(339 - 53 of 2268) GB:U02188(1 - 287 of 287)
MG328	412933	410666	GB:X62467_1	protein V (fcrV) {Streptococcus sp.}	27.5434	48.3871	MG328(817 - 462 of 2268) GB:U02203(1 - 356 of 356)
MG330	414975	414325	SP:P38493	cytidylate kinase (cmk) {Bacillus subtilis}	40.3756	61.0329	MG330(537 - 226 of 651) GB:U02241(1 - 312 of 314)
MG334	419480	416970	SP:Q05873	valyl-tRNA synthetase (valS) {Bacillus subtilis}	38.5629	60.5988	MG334(1109 - 781 of 2511) GB:U02202(1 - 330 of 330)
MG334	419480	416970	SP:Q05873	valyl-tRNA synthetase (valS) {Bacillus subtilis}	38.5629	60.5988	MG334(2400 - 2511 of 2511) GB:U02249(1 - 112 of 305)
MG335	420045	419473	SP:P38424	hypothetical protein (SP:P38424) {Bacillus subtilis}	34.5238	61.3095	MG335(1 - 95 of 573) GB:U02190(200 - 294 of 294)
MG336	421467	422690	GB:U00013_6	nitrogen fixation protein (nifS) {Mycobacterium leprae}	26.2295	47.2678	MG336(990 - 719 of 1224) GB:U02256(1 - 272 of 272)
MG337	422697	423110	-	-	-	-	MG337(414 - 151 of 414) GB:U01709(35 - 297 of 297)
MG338	426915	423103	-	-	-	-	MG338(1 - 251 of 3813) GB:U02269(65 - 315 of 315)
MG338	426915	423103	-	-	-	-	MG338(1304 - 917 of 3813) GB:U02221(1 - 388 of 388)
MG338	426915	423103	-	-	-	-	MG338(3342 - 3067 of 3813) GB:U01809(1 - 276 of 276)

MG338	426915	423103	-	-	-	-	MG338(3772 - 3813 of 3813) GB:U01709(1 - 42 of 297)
MG339	428115	427096	GB:L25893_1	recombination protein (recA) {Staphylococcus aureus}	46.5986	69.3878	MG339(372 - 93 of 1020) GB:U01704(1 - 279 of 279)
MG340	434458	430583	SP:P00577	DNA-directed RNA polymerase beta' chain (rpoC) {Escherichia coli}	44.4828	66.0345	MG340(1294 - 999 of 3876) GB:X61534(1 - 295 of 295)
MG340	434458	430583	SP:P00577	DNA-directed RNA polymerase beta' chain (rpoC) {Escherichia coli}	44.4828	66.0345	MG340(1519 - 1289 of 3876) GB:X61528(1 - 231 of 231)
MG340	434458	430583	SP:P00577	DNA-directed RNA polymerase beta' chain (rpoC) {Escherichia coli}	44.4828	66.0345	MG340(3444 - 3083 of 3876) GB:U02169(1 - 361 of 361)
MG340	434458	430583	SP:P00577	DNA-directed RNA polymerase beta' chain (rpoC) {Escherichia coli}	44.4828	66.0345	MG340(3772 - 3876 of 3876) GB:U01766(1 - 105 of 467)
MG340	434458	430583	SP:P00577	DNA-directed RNA polymerase beta' chain (rpoC) {Escherichia coli}	44.4828	66.0345	MG340(426 - 66 of 3876) GB:U01797(1 - 361 of 361)
MG341	438640	434471	GB:L24376_3	RNA polymerase beta subunit (rpoB) {Bacillus subtilis}	46.5338	67.5043	MG341(1 - 107 of 4170) GB:U02230(217 - 323 of 323)
MG341	438640	434471	GB:L24376_3	RNA polymerase beta subunit (rpoB) {Bacillus subtilis}	46.5338	67.5043	MG341(1932 - 1595 of 4170) GB:U01737(1 - 338 of 338)
MG341	438640	434471	GB:L24376_3	RNA polymerase beta subunit (rpoB) {Bacillus subtilis}	46.5338	67.5043	MG341(2833 - 3201 of 4170) GB:U01735(1 - 369 of 369)
MG342	439236	438733	-	-	-	-	MG342(381 - 504 of 504) GB:U02230(1 - 124 of 323)
MG342	439236	438733	-	-	-	-	MG342(386 - 65 of 504) GB:U02231(1 - 322 of 322)

MG343	440355	439318	-	-	-	-	MG343(108 - 452 of 1038) GB:U01811(1 - 345 of 345)
MG344	441180	440362	GP:U17036_2	lipase-esterase (lip1) {Mycoplasma mycoides}	26.6667	47.5	MG344(575 - 767 of 819) GB:U02222(1 - 193 of 193)
MG345	443878	441194	SP:P00956	isoleucyl-tRNA synthetase (ileS) {Escherichia coli}	33.2963	56.2708	MG345(1115 - 782 of 2685) GB:U02196(1 - 334 of 334)
MG345	443878	441194	SP:P00956	isoleucyl-tRNA synthetase (ileS) {Escherichia coli}	33.2963	56.2708	MG345(1811 - 2134 of 2685) GB:U02254(1 - 324 of 324)
MG348	446165	445200	-	-	-	-	MG348(166 - 459 of 966) GB:U01781(1 - 292 of 292)
MG352	450222	450719	GB:U11883_2	hypothetical protein (GB:U11883_2) {Bacillus subtilis}	33.3333	56.7901	MG352(366 - 498 of 498) GB:U02237(1 - 133 of 310)
MG353	451048	450722	-	-	-	-	MG353(327 - 153 of 327) GB:U02237(136 - 309 of 310)
MG357	455947	454769	GB:L17320_2	acetate kinase (ackA) {Bacillus subtilis}	42.6735	65.5527	MG357(342 - 131 of 1179) GB:X61531(1 - 211 of 211)
MG358	456590	457369	GB:M21298_1	Holliday junction DNA helicase (ruvA) {Escherichia coli}	26.2411	42.5532	MG358(350 - 87 of 780) GB:U02233(1 - 265 of 265)
MG361	459615	460100	SP:P29394	ribosomal protein L10 (rpL10) {Thermotoga maritima}	29.8137	61.4907	MG361(274 - 486 of 486) GB:U02206(1 - 213 of 345)
MG362	460126	460491	SP:P02394	ribosomal protein L7/L12 ('A' type) (rpL7/L12) {Bacillus subtilis}	47.5	70	MG362(1 - 107 of 366) GB:U02206(239 - 345 of 345)
MG365	461682	462614	GB:X63666_2	methionyl-tRNA formyltransferase (fmt) {Escherichia coli}	24.43	50.8143	MG365(292 - 1 of 933) GB:U02238(1 - 292 of 349)

MG368	466410	465427	GB:M96793_1	fatty acid/phospholipid synthesis protein (plsX) {Escherichia coli}	28.972	52.3364	MG368(227 - 1 of 984) GB:U01791(1 - 227 of 326)
MG369	468083	466413	-	-	-	-	MG369(1146 - 1446 of 1671) GB:U01763(1 - 300 of 300)
MG370	469123	468155	SP:P23851	hypothetical protein (SP:P23851) {Escherichia coli}	26.9531	48.8281	MG370(240 - 599 of 969) GB:U02220(1 - 360 of 360)
MG371	470084	469113	GB:D26185_10	hypothetical protein (GB:D26185_10) {Bacillus subtilis}	25.8065	47.0046	MG371(349 - 689 of 972) GB:U02263(1 - 341 of 341)
MG374	472891	472070	-	-	-	-	MG374(1 - 178 of 822) GB:U02250(159 - 337 of 337)
MG375	474578	472887	GB:M36594_1	threonyl-tRNA synthetase (thrSv) {Bacillus subtilis}	38.7097	60.7527	MG375(1048 - 1389 of 1692) GB:U02130(1 - 342 of 342)
MG375	474578	472887	GB:M36594_1	threonyl-tRNA synthetase (thrSv) {Bacillus subtilis}	38.7097	60.7527	MG375(1530 - 1692 of 1692) GB:U02250(1 - 163 of 337)
MG378	477139	475529	SP:P35868	arginyl-tRNA synthetase (argS) {Corynebacterium glutamicum}	33.6406	56.9124	MG378(1364 - 1047 of 1611) GB:U01740(1 - 319 of 319)
MG378	477139	475529	SP:P35868	arginyl-tRNA synthetase (argS) {Corynebacterium glutamicum}	33.6406	56.9124	MG378(765 - 456 of 1611) GB:U02168(1 - 309 of 309)
MG379	477168	479003	GB:L10328_106	glucose inhibited division protein (gidA) {Escherichia coli}	40.7346	61.9366	MG379(900 - 1184 of 1836) GB:U01812(1 - 285 of 285)
MG385	484699	483992	-	-	-	-	MG385(234 - 6 of 708) GB:U02112(1 - 229 of 229)
MG385	484699	483992	-	-	-	-	MG385(523 - 708 of 708) GB:U02239(1 - 186 of 320)

MG385	484699	483992	-	-	-	-	MG385(528 - 259 of 708) GB:U02246(1 - 270 of 270)
MG386	489552	484705	GB:U11381_1	cytadherence-accessory protein (hmw1) {Mycoplasma pneumoniae}	31.1755	49.4037	MG386(1294 - 1628 of 4848) GB:U02175(1 - 335 of 335)
MG386	489552	484705	GB:U11381_1	cytadherence-accessory protein (hmw1) {Mycoplasma pneumoniae}	31.1755	49.4037	MG386(2274 - 1991 of 4848) GB:X61519(1 - 283 of 284)
MG386	489552	484705	GB:U11381_1	cytadherence-accessory protein (hmw1) {Mycoplasma pneumoniae}	31.1755	49.4037	MG386(3247 - 3420 of 4848) GB:U02126(1 - 174 of 174)
MG386	489552	484705	GB:U11381_1	cytadherence-accessory protein (hmw1) {Mycoplasma pneumoniae}	31.1755	49.4037	MG386(3842 - 4196 of 4848) GB:U02192(1 - 355 of 355)
MG386	489552	484705	GB:U11381_1	cytadherence-accessory protein (hmw1) {Mycoplasma pneumoniae}	31.1755	49.4037	MG386(767 - 1281 of 4848) GB:U02245(2 - 515 of 515)
MG388	491004	490702	GB:U00016_19	hypothetical protein (GB:U00016_19) {Mycobacterium leprae}	30.9278	56.701	MG388(285 - 1 of 303) GB:U02265(1 - 285 of 339)
MG389	491530	491150	-	-	-	-	MG389(320 - 129 of 381) GB:U01813(1 - 192 of 192)
MG390	493516	491537	SP:P37608	lactococcin transport ATP-binding protein (lcnDR3) {Lactococcus lactis}	22.3421	46.5331	MG390(1395 - 1744 of 1980) GB:U02218(1 - 350 of 350)
MG390	493516	491537	SP:P37608	lactococcin transport ATP-binding protein (lcnDR3) {Lactococcus lactis}	22.3421	46.5331	MG390(1400 - 1174 of 1980) GB:U02248(1 - 227 of 227)
MG391	494967	493627	GB:D17450_1	aminopeptidase {Mycoplasma salivarium}	41.2921	60.3933	MG391(1 - 217 of 1341) GB:U02268(256 - 472 of 472)
MG391	494967	493627	GB:D17450_1	aminopeptidase {Mycoplasma salivarium}	41.2921	60.3933	MG391(412 - 735 of 1341) GB:U01801(1 - 324 of 324)

MG391	494967	493627	GB:D17450_1	aminopeptidase {Mycoplasma salivarium}	41.2921	60.3933	MG391(412 - 735 of 1341) GB:U01802(1 - 324 of 324)
MG392	496615	494987	GB:L10132_2	heat shock protein (groEL) {Bacillus stearothermophilus}	51.5209	71.4829	MG392(1394 - 1629 of 1629) GB:U02268(1 - 236 of 472)
MG392	496615	494987	GB:L10132_2	heat shock protein (groEL) {Bacillus stearothermophilus}	51.5209	71.4829	MG392(181 - 1 of 1629) GB:U02252(1 - 181 of 296)
MG393	496960	496631	GB:D17398_1	heat shock protein 60-like protein (PggroES) {Porphyromonas gingivalis}	39.5604	54.9451	MG393(330 - 231 of 330) GB:U02252(197 - 296 of 296)
MG394	498306	497089	SP:P06192	serine hydroxymethyltransferase (glyA) {Salmonella typhimurium}	55.303	70.7071	MG394(328 - 683 of 1218) GB:U02131(1 - 356 of 356)
MG395	499890	498319	-	-	-	-	MG395(457 - 116 of 1572) GB:U02260(1 - 342 of 342)
MG395	499890	498319	-	-	-	-	MG395(763 - 979 of 1572) GB:X61530(1 - 217 of 217)
MG399	503976	502831	SP:P33253	ATP synthase beta chain (atpD) {Mycoplasma gallisepticum}	80.9524	89.418	MG399(447 - 852 of 1146) GB:U01752(1 - 406 of 406)
MG400	505099	504263	SP:P33257	ATP synthase gamma chain (atpG) {Mycoplasma gallisepticum}	37.9433	62.0567	MG400(160 - 711 of 837) GB:U01703(1 - 552 of 552)
MG401	506655	505102	SP:P33252	ATP synthase alpha chain (atpA) {Mycoplasma gallisepticum}	63.3911	79.5761	MG401(973 - 1554 of 1554) GB:U01727(1 - 583 of 598)
MG405	509012	508137	GB:X64256_2	adenosinetriphosphatase (atpB) {Mycoplasma gallisepticum}	36.4261	63.9175	MG405(75 - 1 of 876) GB:U01728(1 - 75 of 299)
MG406	509319	508981	SP:P15362	transport system permease protein P69 (P69) {Mycoplasma hyorhinis}	40	57.1429	MG406(339 - 84 of 339) GB:U01728(44 - 299 of 299)

MG410	513042	512056	GB:L10328_89	peripheral membrane protein B (pstB) {Escherichia coli}	50.813	70.3252	MG410(301 - 941 of 987) GB:U01707(1 - 640 of 640)
MG411	514991	513030	GB:X75297_1	periplasmic phosphate permease homolog (AG88) {Mycobacterium tuberculosis}	30.7692	56.2753	MG411(406 - 632 of 1962) GB:U01746(1 - 227 of 229)
MG412	516124	514994	-	-	-	-	MG412(252 - 1 of 1131) GB:U01702(1 - 252 of 313)
MG412	516124	514994	-	-	-	-	MG412(675 - 563 of 1131) GB:U02101(1 - 113 of 113)
MG413	518389	516248	GB:L22432_4	hypothetical protein (GB:L22432_4) {Mycoplasma capricolum}	25	54.1667	MG413(1179 - 701 of 2142) GB:U01699(1 - 480 of 480)
MG413	518389	516248	GB:L22432_4	hypothetical protein (GB:L22432_4) {Mycoplasma capricolum}	25	54.1667	MG413(1535 - 1230 of 2142) GB:U01804(1 - 305 of 305)
MG414	519355	516248	-	-	-	-	MG414(438 - 154 of 917) GB:U01695(1 - 285 of 285)
MG416	521414	520371	-	-	-	-	MG416(1 - 39 of 1044) GB:U01744(580 - 618 of 620)
MG416	521414	520371	-	-	-	-	MG416(7 - 351 of 1044) GB:U02102(1 - 345 of 345)
MG418	522314	521877	SP:P02410	ribosomal protein L13 (rpL13) {Escherichia coli}	41.3043	70.2899	MG418(321 - 438 of 438) GB:U01744(1 - 118 of 620)
MG421	526696	524153	SP:P07671	excinuclease ABC subunit A (uvrA) {Escherichia coli}	47.7541	68.5579	MG421(1693 - 1393 of 2544) GB:X61514(1 - 301 of 301)
MG422	529493	526989	-	-	-	-	MG422(2274 - 2101 of 2505) GB:U02117(1 - 174 of 174)

MG422	529493	526989	-	-	-	-	MG422(2439 - 2505 of 2505) GB:U02172(1 - 67 of 318)
MG422	529493	526989	-	-	-	-	MG422(35 - 1 of 2505) GB:U02228(1 - 35 of 304)
MG423	531216	529534	-	-	-	-	MG423(1434 - 1197 of 1683) GB:X61510(1 - 238 of 238)
MG423	531216	529534	-	-	-	-	MG423(161 - 413 of 1683) GB:X61524(1 - 252 of 255)
MG423	531216	529534	-	-	-	-	MG423(1683 - 1455 of 1683) GB:U02228(76 - 304 of 304)
MG425	531668	533014	SP:P23304	ATP-dependent RNA helicase (deaD) {Escherichia coli}	32.4121	58.0402	MG425(989 - 769 of 1347) GB:U01805(1 - 220 of 220)
MG431	538290	537559	GB:L27492_1	triosephosphate isomerase (tim) {Thermotoga maritima}	39.7541	61.8852	MG431(463 - 732 of 732) GB:U02109(1 - 270 of 277)
MG437	542067	542981	GB:M11330_1	CDP-diglyceride synthetase (cdsA) {Escherichia coli}	38.0165	55.3719	MG437(679 - 378 of 915) GB:U02189(2 - 303 of 303)
MG441	546707	546300	-	-	-	-	MG441(20 - 318 of 408) GB:U02128(1 - 299 of 299)
MG447	552444	550804	GB:L08897_1	hypothetical protein (GB:L08897_1) {Mycoplasma gallisepticum}	34.058	55.0725	MG447(319 - 645 of 1641) GB:U01788(1 - 327 of 327)
MG451	555612	554431	SP:P13927	elongation factor TU (tuf) {Mycoplasma genitalium}	100	100	MG451(927 - 586 of 1182) GB:U02255(1 - 342 of 342)
MG453	556435	557310	GB:L12272_1	UDP-glucose pyrophosphorylase (gtaB) {Bacillus subtilis}	48.0287	65.233	MG453(491 - 181 of 876) GB:U02258(1 - 311 of 311)

MG455	557724	558944	GB:M77668_1	tyrosyl tRNA synthetase (tyrS) {Bacillus stearothermophilus}	38.539	61.7128	MG455(604 - 362 of 1221) GB:U02247(5 - 247 of 247)
MG456	559941	558940	-	-	-	-	MG456(256 - 568 of 1002) GB:U01790(1 - 312 of 312)
MG458	563307	562783	SP:Q02522	hypoxanthine-guanine phosphoribosyltransferase (hpt) {Lactococcus lactis}	38.3721	66.8605	MG458(295 - 24 of 525) GB:U02193(1 - 272 of 272)
MG459	563818	563312	GB:M64978_2	surface exclusion protein (prgA) (Plasmid pCF10) {Enterococcus faecalis}	28.3582	49.2537	MG459(330 - 1 of 507) GB:U01725(1 - 330 of 638)
MG460	563991	564926	SP:P33572	L-lactate dehydrogenase (ldh) {Mycoplasma hyopneumoniae}	50.3226	67.7419	MG460(1 - 136 of 936) GB:U01725(503 - 638 of 638)
MG462	567638	566187	GB:M55072_1	glutamyl-tRNA synthetase (gltX) {Bacillus stearothermophilus}	42.887	65.272	MG462(1452 - 1081 of 1452) GB:U02122(9 - 379 of 379)
MG463	568404	567628	GB:D26185_105	high level kasgamycin resistance (ksgA) {Bacillus subtilis}	35.6164	53.8813	MG463(777 - 409 of 777) GB:U01719(36 - 405 of 405)
MG467	570988	570056	GB:X75422_1	heterocyst maturation protein (devA) {Anabaena sp.}	39.899	63.1313	MG467(40 - 352 of 933) GB:U01741(1 - 313 of 313)
MG469	578578	577268	SP:P34028	chromosomal replication initiator protein (dnaA) {Spiroplasma citri}	30.9469	57.2748	MG469(845 - 547 of 1311) GB:U02259(1 - 299 of 299)
MG469	578578	577268	SP:P34028	chromosomal replication initiator protein (dnaA) {Spiroplasma citri}	30.9469	57.2748	MG469(855 - 1206 of 1311) GB:U02145(1 - 352 of 352)

Table 1(d)

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MG417 MORF-20402

MG418 MORF-20052

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MG455 MORF-20423 MORF-20424

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MG457 MORF-20069 MORF-20425

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MG467 MORF-20432

MG468 MORF-20283

MG469 MORF-20434

MG470 MORF-20071 MORF-20435

Table 2

UID	end5	end3	gene_len
MG016	19253	19756	504
MG017	19825	20352	528
MG027	30092	30544	453
MG028	30547	31149	603
MG064	74066	77683	3618
MG076	102870	102457	414
MG105	133569	134168	600
MG117	143310	143951	642
MG147	186138	187262	1125
MG185	211445	213547	2103
MG186	216017	216766	750
MG199	237094	236594	501
MG202	239826	240191	366
MG207	247523	247906	384
MG211	250997	251437	441
MG223	268011	269243	1233
MG230	276166	276624	459
MG236	280663	281082	420
MG241	286884	288743	1860
MG243	290976	291323	348
MG246	293936	294778	843
MG256	306819	307586	768
MG267	325157	324813	345
MG279	341181	340528	654
MG284	346853	347248	396
MG286	348260	348847	588
MG296	364414	364028	387
MG306	377974	376796	1179
MG321	402922	400121	2802
MG331	415622	414987	636
MG333	416716	416339	378
MG349	446576	447787	1212
MG350	447790	448722	933
MG354	451197	451607	411
MG366	462619	464619	2001
MG372	471234	470080	1155
MG373	472066	471224	843
MG376	474892	474581	312
MG377	475479	474901	579
MG381	479570	480223	654
MG397	502420	500723	1698

MG415	520238	519929	310
MG419	523215	522355	861
MG427	533270	533692	423
MG428	533806	534318	513
MG436	542092	541739	354
MG439	545378	544563	816
MG440	546154	545381	774
MG449	553295	552864	432
MG450	554269	553559	711
MG452	555665	556447	783
MG468	318330	319202	873

The Nucleotide Sequence of the Mycoplasma genitalium Genome

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While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention.

All patents, patent applications and publications recited herein are hereby incorporated by reference.

TABLE 3

Whole Genome Sequencing Strategy	
Stage	**Description**
Random small insert and large insert library construction	Randomly shear genomic DNA on the order of 2 kb and 15-20 kb, respectively
Library plating	Maximize random selection of small insert and large insert clones for template production
High-throughput DNA sequencing	Sequence xxx,xxx templates from both ends (>99% genome coverage)
Assembly (**TIGR Assembler, GRASTA**)	Assembly of sequence fragments into contigs
Gap closure	
a. Physical gaps	Order all contigs into a circular genome and provide templates for closure of all physical gaps
b. Sequence gaps	Complete the genome by primer walking
Editing	Visual inspection and resolution of all sequence ambiguities when possible, including frameshifts
Annotation	Identification and description of all ORF's, putative identification, role assignments

TABLE 4

Computer simulation of random sequencing experiments where L = 580,000 and w = 400.

Clones sequenced (n)	Percent of genome unsequenced	Base pairs unsequenced	Number of double strand gaps	Average gap length (bp)
1000	50.18	291014	501	580
2000	25.18	146016	503	289
4000	6.34	36759	253	145
6000	1.60	9254	97	96
7250	0.67	3886	48	80
8000	0.40	2330	32	72
10000	0.10	586	10	59